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## 第 3 巻 目 次　　Contents of Vol. 3

### 第 1 号　No. 1 (Issued 20th December, 1960)


大泰司 諭　　Pages

毛皮獣の被毛，特に Nutria の被毛 …… 1–183
(Ôtaishi, Satoru: A Study on the Pelage of the Nutria referring to the Pelage-Chang of Fur Animals in General)

Anagama, Yûzô & Kami, Takayasu

Die Anwendungsmöglichkeiten der Formoltitration in der Molkerei-praxis …… 185–189
(穴釜雄三・上　隆保：酪農の実地におけるフォルモール滴定法の適用可能性)

Anagama, Yûzô & Kami, Takayasu

Jahreszeitliche Schwankungen des Gehaltes an Kasein und Molkeneiweiss der Kuhmilch …… 191–196
(穴釜雄三・上　隆保：牛乳のカゼイン及び乳清蛋白質含量の季節的変動)

Fujii, Shunsaku

A Single Differential Staining Method for Vaginal Smears …… 197–201
(藤井俊策：膣脂垢の簡易鑑別染色法)

Irie, Takaaki

The Growth of the Fish Otolith …… 203–229
(入江隆明：魚類耳石の生長)


### 第 2 号　No. 2 (Issued 20th December, 1961)


Kakuda, Shunpei

Breaking Strength of a Knot of Fishing Net as Affected by the Angle between Legs of Mesh. …… 231–245
(角田俊平：引張り方向が網糸の結節強度に及ぼす影響)

松平 康雄・小山 治行・遠藤 拓郎

福山港水域の海況の概要 …… 247–296
(Matsudaira, Yasuo, Koyama, Haruyuki & Endo, Takuro: Hydrographic Conditions of Fukuyama Harbor)

TAKI, Iwao

On Two New Eledonid Octopods from the Antarctic Sea ..... 297–321
(滝　巌：南極洋産タコ（エレドーネ類）2新種について)

MURAKAMI, Yutaka

Studies on the Winter Eggs of the Water Flea, *Moina macrocopa* STRAUS ..... 323–349
(村上豊：タマミジンコの冬卵に関する研究)

ISHIKAWA, Yasutaro

Some Considerations on Diving of Whales ..... 351–374
(石川安太郎：鯨の潜水に関する考察)

NAKAMURA Tsunenori

Cytological and Histological Studies on Abnormal Follicles of Mature Mice ..... 375–437
(中村経紀：成熟マウスの異常濾胞に関する細胞学的組織学的研究)

WATANABE, Moriyuki

Experimental Studies on the Artificial Insemination of Domestic Ducks with Special Reference to the Production of Mule-Ducks ..... 439–485
(渡辺守之：あひるの人工授精に関する実験的研究，特に土蕃鴨の生産について)

ANAGAMA, Yûzô & KAMI Takayasu

Studien über Zitronensäure in Kuhmilch. I. Jahreszeitliche Schwankungen des Gehaltes an Zitronensäure der Kuhmilch ..... 487–492
(穴釜雄三・上　隆保：牛乳の枸櫞酸含量の季節的変動)

FUJII, Shunsaku

Light-Microscopic Studies on Changes in the Ultrastructural Density of Skeletal Muscle Fibers Caused by Thermal Treatment ..... 493–501
(藤井俊策：熱処理による骨格筋の光学顕微鏡下の超構密度の変化)

橋本秀夫

畜産食品に含まれる腸球菌群に関する衛生学的研究，特に汚染指標としての意義について ..... 503–566
(HASHIMOTO, Hideo : Hygienic Studies on Enterococcus Group in Milk, Meat and Their Products, Especially on Significance as an Indicator of Pollution)

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List of Papers by the Staff of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, Published during January, 1959 to December, 1960.

Department of Fisheries ..... i–ii
Department of Animal Husbandry ..... ii–iii

# 毛皮獣の被毛の研究，特に Nutria の被毛について

大　泰　司　　諭

広島大学水畜産学部畜産学科

# A Study on the Pelage of the Nutria referring to the Pelage-Chang of Fur Animals in general

Satoru ÔTAISHI

*Department of Animal Husbandry, Fadulty of Fisheries and Animal Husbandry, Hiroshima University, Fnkvyama.*

(Tables 1—104; Text-figs. 1—58; Plates 1—17)

I　序　　論 …… 1
II　資料の採集と調製 …… 5
III　標本の作製と整理 …… 6
IV　実験の方針 …… 8
V　毛の分類 …… 10
1　緒　　言 …… 10
2　毛色の明暗と毛の分類との関係 …… 10
(1) 毛の色調 …… 10
(2) 上毛と下毛の分岐域 …… 11
(3) 上毛の変異 …… 14
3　髄質，皮質，色素の関係 …… 42
4　毛小皮，皮質，髄質の関係 …… 48
5　毛の分類の総括 …… 54
VI　発生と換毛 …… 55
1　毛束と毛群 …… 55
2　換毛の生態 …… 60
3　換毛期の発見 …… 61
4　生毛から成獣の被毛まで …… 73
5　毛　世　代 …… 85
6　換　毛　波 …… 102
7　毛量の変遷 …… 103
VII　成獣被毛の体重別（年齢別）及び性別による差異 …… 108
1　体重別（年齢別）の差異 …… 109
2　性別の差異 …… 111
VIII　成獣被毛の季節的変化 …… 112
1　被毛の年間変遷 …… 113
2　寒暖2季の被毛繊度の比較 …… 129
IX　結論及び摘要 …… 140
X　SUMMARY AND CONCLUSIONS …… 143
XI　文　　献 …… 147

## I　序　　論

毛皮獣の被毛に関しては人類は有史以来長年に亘り知識を有しているが，毛皮使用の実際の経験を基礎として得られた名人級の知識であつて欧米においても被毛の学理的研究は極め

て少ない．これ毛皮の使用は常に時の流行によつて支配されて変化只ならず学理的研究がこれに追いつかない状態が繰り返されているからである．近来は野生毛皮の使用は年々減少し養殖毛皮がこれに代り，漸次毛皮の生産に人工が加わるに至つた．ここにおいて早晩毛皮の生産並に使用について学理的研究が基礎とならざるを得ない状態に達したのである．

筆者は1933年以来動物被毛の研究に従事して来たが現在最も入手し易い毛皮獣についてその胎児及び幼獣時代の生毛(うぶげ)の発生の変遷を経て，成獣の被毛の換毛過程を調査研究し，剝皮適期を究めんとして，ここに Nutria を用いて研究をした．

Nutria は学名を *Myodastor Coypus* (*Molina*)，日本名をかいりねづみ(海狸鼠)ということもある．南米の南緯20度以南のアンデス山系の両斜面主としてラプラタ河を中心とする亜熱帯地の河川，湖沼の低湿地に棲息する水陸両棲の嚙歯類で完全な菜食の動物で，この毛皮は優良品として有名である．アルゼンチン政府の統計によれば毛皮生産高は次のようである．

1918……338, 694 kg　　1 kg は毛皮の 4 乃至 5 枚に当る．
1923……157, 401 〃
1928…… 27, 998 〃
1930…… 9, 253 〃

野獣は濫獲のために遂年激減し現在では捕獲を制限すると共に海外に動物の移動を厳禁しており，又同国ではこの動物の家畜化に成功して野生のものに勝る改良品種 Las Tres Lagunas 種を固定し繁殖している．

一方毛皮需要国である欧米では1882年に仏国に数頭が輸入され飼育が始められたが，第一次世界大戦で一時野放しにされたものが狩猟の獲物に化していた．1930年頃から独逸，加奈陀，北米合衆国で試験的な飼育が始められて第二次大戦中には中北欧諸国にやや大量の増殖が試みられたが，打続く戦禍にあい激減すると共に無秩序な近親繁殖のために品質と体位は下向して，戦後はその産額，品質ともに見るべきものがない．1951年の独逸，スエーデン，デンマーク，ノルエー諸国の総計頭数は 1.4 万頭内外にすぎない．ことに北欧，加奈陀等の酷寒地の飼育では充分な繁殖をうるには大きな犠牲が払われている．

従つて各国の飼育経験者はこの動物の繁殖育成には温暖な営巣場所と良皮をうるには清冷な流水，泉水が要ると結論している．すでに南米の原産地では良品種を世界の毛皮市場に大量に供給しており，北米産の mink の毛皮と比肩する傾向にある．

尚北米ではかって戦後の劣悪な Nutria を見限ぎり，国産 mink の飼育に転じていた者の内にもアルゼンチンから Nutria の改良種を輸入してカリフオルニアの暖地に大規模な飼育経営が始められた (Southworth 1954)．

わが国の Nutria 飼育史は昭和 6 年 6 月 (1931. 6) に高松宮が独逸から数頭携帯されたが繁殖なく，同年10月仏国から福井文吉氏が数頭輸入したがこれもまた繁殖なく，昭和 8 年 (1933) に川島惇三氏が一番を輸入しる 3 頭の繁殖をみたのが日本での Nutria 第一号といえよう．

昭和15年 1 月 (1940) に川島氏は 75 番 (150頭) を北米合衆国から輸入し，その内47頭は陸軍獣医学校に入れ緒方直光氏の研究資料として，またその一部は川島氏経営の日南商会で飼育され残余が一般に分譲された (栗原1948)．

かくて戦時中に蛋白と毛皮の資源涸渇に便乗して増殖された結果，昭和19年 (1944) には約30, 000頭を数えた (内田1944)．

その後わが国の飼育成績は関東以北ことに北海道等の寒冷地では欧米の例に似て繁殖に多くの犠牲が払われた．さらに戦後食い余の僅か数百頭を基にして種畜景気にあふられながら，昭和30年（1955）には中国地方以西のみでも約2万頭に殖えたといわれるが，この極端な近親繁殖の結果は，授胎不能，早産，出産児の発育不全など繁殖の不良の素因をかもして一般に体位並びに毛皮の品位は落ちている．筆者は日本ヌートリヤ毛皮生産同志会主事の小畠真氏と共に昭和29年12月—30年3月（1954. 12—1955. 3），昭和30年12月—31年3月（1955. 12—1956. 3）の2季に亘って Nutria の生畜589頭（産地：岡山県，広島県）について調査して次の表をえた．

第1表　Nutria の体重区分による度数表（屠殺処分せしものより）

| 生体量（貫） | 0.2～0.3 | 0.3～0.4 | 0.4～0.5 | 0.5～0.6 | 0.6～0.7 | 0.7～0.8 | 0.8～0.9 | 0.9～1.0 | 1.0～1.1 |
|---|---|---|---|---|---|---|---|---|---|
| 頭　数 | 1 | 6 | 14 | 40 | 56 | 61 | 44 | 57 | 58 |

| 1.1～1.2 | 1.2～1.3 | 1.3～1.4 | 1.4～1.5 | 1.5～1.6 | 1.6～1.7 | 1.7～1.8 | 1.8～1.9 | 1.9～2.0 | 2.0～2.1 | 合　計 |
|---|---|---|---|---|---|---|---|---|---|---|
| 47 | 49 | 44 | 37 | 26 | 28 | 12 | 5 | 2 | 2 | 589 |

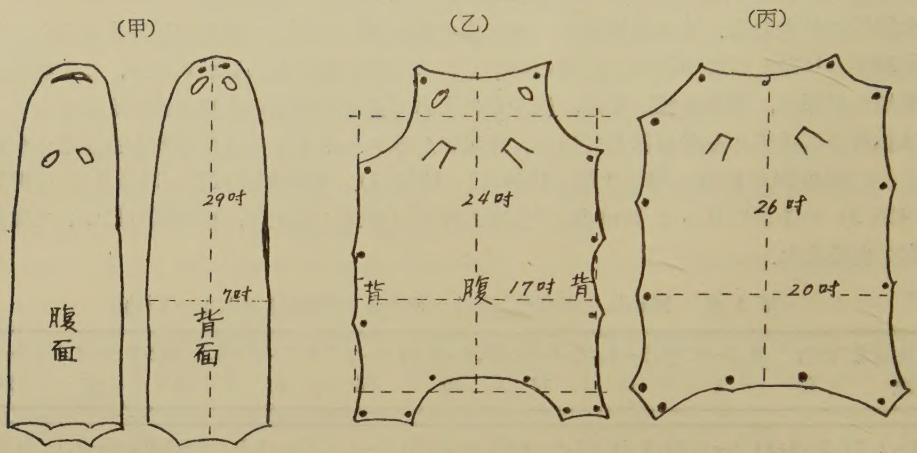
第2表　Nutria の体重，月齢区分による度数表（屠殺処分のものと，生体観察のものと混在）

| 生体量（貫）／♂♀別／月齢 | 0.7 | | 0.7～0.9 | | 0.9～1.1 | | 1.1～1.3 | | 1.3～1.6 | | 1.6～2.6 | | 小　計 | | 合　計 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂＋♀ |
| 9 | | | 5 | 5 | | | | | | | | | 5 | 5 | 10 |
| 10 | | | 1 | 2 | 2 | 1 | 1 | | | | | | 4 | 3 | 7 |
| 11 | | | | | | | | | | | | | | | |
| 12 | 2 | 1 | 2 | | 2 | 1 | | | | 1 | | | 6 | 3 | 9 |
| 13 | | | 1 | 1 | | 1 | | | | | | | 1 | 2 | 3 |
| 14 | | | | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | | | | |
| 16 | | | | | | | | | | 2 | | | | 2 | 2 |
| 17 | | | | | | 1 | | 1 | | | | | | 2 | 2 |
| 18 | | | 1 | 1 | 1 | | | 1 | | | | | 2 | 2 | 4 |
| 19 | | | | | | | | | 1 | | | | 1 | | 1 |
| 23 | | | | | | | | | 1 | 1 | | | 1 | 1 | 2 |
| 30 | | | | | | | 1 | | 1 | 2 | | | 1 | 3 | 4 |
| 31 | | | | | | | 1 | | | 1 | | | | 2 | 2 |
| 32 | | | | | | | | | | 1 | | | | 1 | 1 |
| 36以上 | | | | | | | | | | | 6 | 6 | 6 | 6 | 12 |
| | 2 | 1 | 10 | 9 | 5 | 4 | 1 | 4 | 3 | 8 | 6 | 6 | 27 | 32 | 59 |

また干皮の調査には図の甲，乙の場合を選んで扱つたが生体量1.5貫の動物からえた干皮は甲で29吋×7吋，乙では24吋×17吋のものをえた．北欧，加奈陀の例では毛皮剝皮の月齢を18ケ月以上にみて，丙図のような調製干皮で約20吋×26吋である．他方アルゼンチンの改良品種では生体量4.2貫—4.8貫（35—40磅）で丸剝素干皮（甲図）の頭鼻端から尾根まで32吋—40吋を報じている．（Thompson 1954）

これと比較するにわが国産，欧米産が如何に体位において劣つているかが伺われる．また欧洲，加奈陀産毛皮の例では腹部綿毛は約2糎（$\frac{3}{4}$吋）を報じている（Hodgson）．

第 1 図




甲図. 丸剝素干皮，肉面を外にして一端を円く縁どった木板を挿入して風干する．
乙図. 剝皮後，口縁鼻部を切りはなし，背線に沿うて背割(セワリ)をして，緊張して，板張りする．(写5図)
丙図. 背割りして板張り風干する．欧米では甲，丙図の如く調製する．

筆者の調査範囲で，わが国のものは腹部綿毛が1.5糎を越えるものは稀れである．さらに綿毛の色相においても良品とされる濃灰色 (dark blue slate color) のものは少く，下級品に属する淡チョコレート灰色 (slight chocolate slate color) 或は最も下級品と見られる帯赤灰色 (redish slate color) が多い．Las Tres Lagunus 種では突然変異として白色 (white color)，黄金色 (golden color) 等がある．わが国産 Nutria は以上のように悪条件に拘らず昭和29年12月—30年2月生産の筆者等の鞣製加工試験毛皮 2,500 枚の内昭和31年1月 (1956. 1) の東京毛皮市場に提出の物は世界毛皮市場水準として取扱はれている事実からしても，わが国内において Nutria 飼育に適地のあることが伺い知られるのである．因にこの毛皮市場に供されたものは岡山県，広島県産で飼育者も毛皮動物に未経験であり．また市街地及びその近郊において種採り本意で飼育されたので，毛皮動物としての管理は考慮されていないものである．加うるに当時は種採り景気は霧散して飼育者の動物放棄が続出する有様であつて前記の調査表で見るように生体量の過少な幼獣まで鞣製試験に提供されたのである．おもうにわが国の中西部の暖地において清冷な水利と豊かな植物性飼料をえて飼養可能の環境を選び，良品種を導入して改良，増産をはかれば多量の良毛皮を内外市場に供給しうるであろう．欧米の Nutria 飼育家はこの動物は他の陸棲の毛皮獣のように年2回の換毛型でなく人毛のように随時換毛するので，飼育管理次第ではかなり大幅な毛皮採拾期間をみとめうるとしている．

一方又以上のように飼育家による Nutria 飼育の体験記録は多いが，まだこれについて科学的な観察の文献は乏しい．

本研究の結果報告に当り，終始御指導を与えられた北海道大学教授先本勇吉博士，校閲の労を煩はし御指導を賜った恩師犬飼哲夫博士に衷心より謝意を表する次第である．

尚本研究を遂行するに当り，多大の御後援を賜った広島大学教授池田実博士，北海道大学教授高畑倉彦博士，松本久喜博士，中村良一博士，広島大学助教授内村操六氏，動物資料

を提供された広島県福山市箕島町農業小畠真氏，文献蒐集に多大の援助を賜った岡山大学教授高橋隆平博士，広島大学教授三村耕博士，助教授藤井俊策博士，四国農業試験場伊藤健次氏，北海道農業試験場西濃高一氏の諸氏に深甚の謝意を表する次第である．

## 序　論　文　献

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内田清之助：昭和19年（1944）農業世界，11月号．

## Ⅱ 資料の採集と調製

1.　3ケ月胎児1腹1頭：　これは母獣を屠殺処分してえた（♂）．10％フオルマリン溶液に浸漬して貯蔵した．

2.　出産後の幼獣1腹：　10月15日生，♂5頭，♀2頭，この内♂5頭を飼育中に，生後2日（10月16日，生体量130瓦），1ケ月（11月14日，生体量450瓦），2ケ月（12月15日，生量体600瓦），3ケ月（1月15日，生体量800瓦），4ケ月（2月15日，生体量1,100瓦）のように毎月，各1頭を屠殺処分して生皮をえて，フオルマリン溶液に貯蔵した．1腹の幼獣を選んだ理由は，同一個体で月別（或る期間毎に）生皮資料をえられないので，別個体で成長期を合わせることと，類似点の多いことを期待した．

3.　生産後の幼獣1腹：　3月25日生，♂2頭，♀1頭，これを飼育中♂2頭は約3ケ月で死亡，♀1頭を頼りに生後1ケ月（4月25日，生体量500瓦），2ケ月（5月25日），3ケ月（6月25日，生体量900瓦），4ケ月（7月25日，生体量1,200瓦），6ケ月（9月25日，生体量1,500瓦），まで採毛を続けたが，8ケ月目に♂を配して同棲させた為め，11月20日（生体量2,500瓦）に先住の♀を咬み殺したので，採毛は中絶された．生皮を採取してフオルマリン溶液に貯蔵した．日数は不足であるがこれを8ケ月の資料に使用した．

4.　冬季に毛皮用として処分した成獣皮（12ケ月以上）の背毛皮の一部を採取した．

| 生体量（貫） | 約1.0 | 約1.5 | 約2.0 |
|---|---|---|---|
| kg | 3.5 | 5.5 | 7.5 |
| ♂（頭） | 5 | 5 | 5 |
| ♀（頭） | 5 | 5 | 5 |

毛皮用原皮の為め腹部毛皮の資料は採取不能，背部毛皮の資料を採取，これ等で牝牡性別，又は体位による差異を調査した．

5.　農家に飼育中の成獣，♂2頭，♀2頭計4頭から冬季（1956，1月28日），夏季（1956，5月30日）に背，腹の毛一撮みを採取し，これを寒暖2季の毛の比較にあてた．生体量♂9.3 kg，7.8 kg，♀5.6 kg，5.3 kg であった．

6.　1ケ年間を通じて月別に成獣生皮を採集することにしたが，ついに8月，9月分の生皮は採集不能であった，その他10ケ月分の生皮をえて月別寒暖の差異の調査にあてた．

以上により生皮から採毛，切片標本をえたのは約50頭分であった．

## Ⅲ 標本の作製と整理

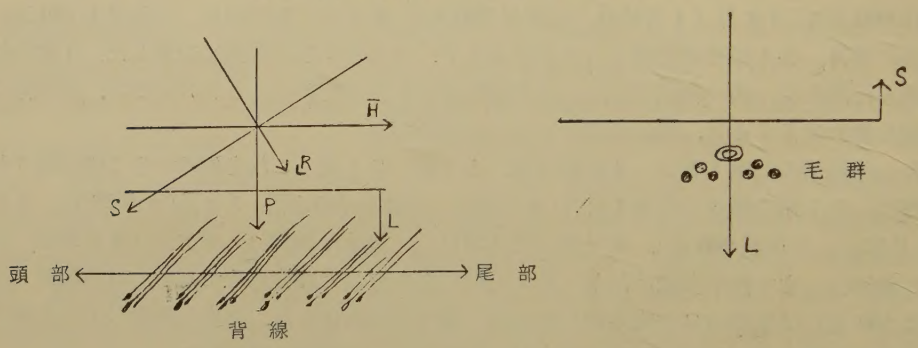
1. 3ケ月胎児からは図（写3図）のように4部位から約 1 cm² の表皮を切り取り常法により，パラフイン包埋後，H̄切片，L 切片，S 切片，ℝ 切片をとりヘマトキシリン，エオヂン染色を行った．又一部はミクロトームで細切せず皮片をそのまま脱水してバルサムで封入し，原色の標本を作った．

2. その他の幼獣，成獣は凡て背腹2カ所から図（写4図）のように生皮を採り各種の切片標本を作った．切片の厚さは切片中に毛幹が含まれるように厚さ 20—40 μ とした．又染色は凡て1と同じである．またℝ切片は連続切片とした．

切片の呼称

H̄切片： 表皮の最上位の水平の切片
ℝ切片： 毛幹に直角な連続切片
L 切片： 背線に沿うて毛幹に平行な切片
S 切片： 背線を横断して毛幹に平行な切片
P 切片： 背線に直角な切片

第 2 図 刀の切断の方向



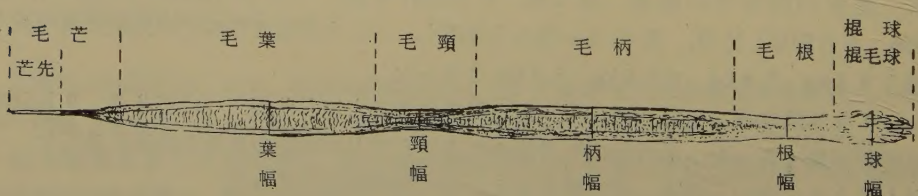

3. ホルマリン漬生皮からの採毛は，生皮を流水によく洗い常温で蒸溜水に浸して，数日放置して毛根を緩めて抜き取り，濾紙に挟んで脱水し，室温に干燥してパラフイン紙に封じた．剪毛した採毛は室温に放置し，干燥してパラフイン紙に封じた．

4. 半裁皮はフオルマリン鞣革として皮面の毛群痕の調査にあてた．

5. Sump により各種被毛の毛小皮の押型及び皮面の毛群の押型を作った．

6. 毛の各部位の呼称と測定位置，毛の各部位の名称は未だ系統的に確立したものがない故に従来の経験により次の如くすることを適当と認めた．

第 3 図 上毛の明葉毛の模写



毛に直角な横断面はほぼ正しい円形又は明らかに長径，短径を決めうる楕円形である．

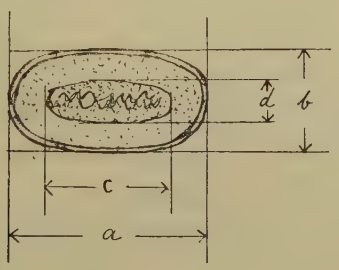
この幅は凡て円形は直径，楕円形は長径の測定値で表わすことにした．毛幹をスライドグラスによこたえ，グリセリンに浸漬してカバーグラスで被うと，視野に現われるのは楕円形の場合は長径である．長径が平面に対して僅かに傾斜すると像の焦点が合わないで，毛幅の両縁が明らかでないが，毛幹の位置の調整は容易であるから楕円の長径の測定は速かに確実にできる．

毛幹に共通な形態的な特徴を認めて，第3図に示すように呼ぶことにした．全毛幹のほぼ中央に細くくびれた部分がある．これを毛頸（モウケイ）と呼び，最も狭い幅を測って頸幅（ケイフク）とする．

この毛頸から前半を毛葉（モウヨウ），その先端を毛芒（モウボウ）と呼ぶ．又毛芒の最先端の部分を芒先（ボウセン）とすることあり．毛葉は上毛と下毛の別なくがいして毛の最も幅の広い部分を含んでいる．この最広部の測定値を葉幅（ヨウフク）とする．さらに毛頸から後半の毛幹は毛根でややくびれて狭くなり後端は粗面の棍状毛球である．この毛幹の後半は毛根までの間に最も平均した毛幅が続いているので，この間で最も広い毛幅を全毛幹を代表する標準測定位としてその測定値を柄幅（ヘイフク（ツカハバ））とし，この間を毛柄（モウヘイ）と呼ぶことにする．

さらに毛根は毛球との中間の最も狭い部分を測り根幅（コンプク）と呼ぶ．なお根球は最もふくれた幅の広い所を測って球幅（キュウフク）とする．また各測定位置の毛髄部の幅を同時に測定することもある．これをその位置の名を冠して髄幅（ズイフク）と呼ぶ．

以上の測定位置は上毛では楕円の長径で下毛では円の直径で表わすことになる．なおL切片に現われて来る毛の縦断面の径はこれを側幅といい，又測定部位によって葉側幅，柄側幅といい，この面を側幅面と呼ぶ．




第4図　毛幹断面模写

a＝長径＝正幅．
b＝短径＝側幅．
c＝髄正幅＝髄幅．
d＝髄側幅．

楕円形の短径の側幅に対して長径を正幅とするが，この正幅の呼称は一般に使用する要はない．R断面が円形の場合はa＝bで，楕円形の場合はa＞bである．

7.　毛原基（Haar Anlage）の発育過程は STÖHR の原語に基いて羽部，上坂等（京都大学）が使用した毛芽期（Haar keim-Stadium），毛栓期（Haarzapfen-），球栓期（Bulbusapfen-），鞘毛期（Scheidenhaar-）の4期として，又この4期の毛原基を毛芽（hair germ）として一括することもある．それ以後，体表に現われて落毛に至る発育過程は，幼毛期（毛頸までの発育），青毛期（毛柄の発育中），成毛期（毛根部の発育中から棍状毛の落毛まで）の3期とし，その期間の呼称を各々幼毛，青毛，成毛，棍状毛（younger hair, young hair, club hair）とし，また幼毛と青毛を一括して発育毛（growing hair）と呼ぶこともある．更に毛原基（anlage），毛母基（matrix），毛包（follicle）は毛の発生基の単位を表わすので，時にこれを混用する場合もあるが，とくに毛母基と毛包は同じ意味に使用している場

合がある.

8. 毛は全毛が暗色で不透明のものと，毛葉が透明（顕微鏡の視野で）で明色の葉明帯 (agouti band) のあるものと，2つの型がある，前者は暗葉毛，後者を明葉毛とする．下毛は凡て暗葉毛で，上毛は明葉毛と暗葉毛の2種類がある，その色調の明さの程度によりM, 2M, 3M, A, 2Aの符号で表わし，これを明度記号とする．その内訳けは次のように分った．

暗葉毛，2A：A： 全毛幹が不透明の暗色で濃褐色→黒褐色．毛葉の中央から毛芒基に及んで黄色を含んでいるが褐色，黒色に被われて暗色で不透明である．毛芒下方に褐色素を透して黄色の現われている淡色の場合をAとする．

明葉毛，M： 毛葉の先端から毛芒まで黄色の明色で褐色素に被われず，透明である．

2M： 毛葉の中央の最も幅の広い部分から毛芒に及んで黄色の明色で透明である．

3M： 毛葉の部分が全体に黄色の明色で透明である．

9. 毛の世代（hair generation）の記号

生毛 $G_1$, 2代後継毛 $G_2$, 3代後継毛…→累代を $G_3$……→$G_n$ とし，2次発生毛（これは小型下毛であるが）は $g_1^1$…… $g_n^n$ とした．右上方の数字は生後副側毛の発育が明了になる生後2ケ月より順次各月別を示す数字で，幾ケ月後生じた副側毛であるかを示す．下方の数字は世代を表わす．

10. 成長記号，毛芽●，幼毛◉，青毛◎，成毛○，の画号と，毛芽C', 幼毛C, 青毛Y, 成毛Oの文字記号で表わした．

11. 発生記号 (development mark)．1毛包に1毛がある場合これを単生毛 (solitary hair) とし，その状態を単生するとした．これをS. 記号で表わす．1毛包に2毛がある場合，対生毛 (couple hairs)，対生する，P. とした．

1毛包に3毛がある場合，連生毛 (trio hairs)，連生する．T. とした．なお対生毛束 (couple hairs bundle)，連生毛束 (trio hairs bundle) 等を用いる．

12. 以上の世代記号，成長記号，発生記号は多く組み合せて使用した．例えば P. O. Y. とせば成毛，青毛の対生するもの，T. O. O. Y., 又は T. 2 O. Y. は成毛2個と青毛1個が連生することである．

13. 毛小皮の形状は HAUSMANN (1924) の分類による coronal 及び imbricate を，上坂等 (1950) の使用した冠状及び瓦列状として引用した．

14. 毛の分類呼称は阿部 (1931) の上毛，下毛を使用する，これは本論中で詳細に述べる．

15. 毛の分類に使用した動物とその記号は第表3のようである．

## Ⅳ 実 験 の 方 針

先ず初めに毛の分類を確立する必要がある．各種の代表的な毛の繊維について毛芒から棍球まで，その全毛を測定して観察する．次に生皮の切片内に含まれている繊維の断片を検鏡してそれ等を比較対照した結果から毛の生態を推測する．調査は背毛が色彩，形態ともに腹毛より鮮明で状態の変化がつかみ易いので主としてこれを標準として調査を進めた．

毛繊維の分類： 多くの哺乳類の被毛は上毛と下毛とのあらまし2つに分つことは容易であるが，両者の間にあって毛葉は下毛に較べてやや幅が広く，毛柄は細く下毛的で中間型のものがある．この中間型の内に上毛と下毛の境界を発見して上毛と下毛の両方に向って調査

第3表

| No. | 資料入手経過 | 性別 ♀♂ | 月齢 月 | 生体量 kg | 動物記号 |
|---|---|---|---|---|---|
| 1 | 屠殺処分（1月） | ♂ | 12以上 | 5.40 | N. A |
| 2 | 屠殺処分（10月） | ♂ | 18以上 | 5.60 | N. B |
| 3 | 1腹出生月別屠殺処分（10月15日生）（10月16日） | ♂ | 生後2日 | 0.13 | K. N. 0 |
| 4 | 〃（11月14日） | ♂ | 1 | 0.45 | K. N. 1 |
| 5 | 〃（12月15日） | ♂ | 2 | 0.60 | K. N. 2 |
| 6 | 〃（1月15日） | ♂ | 3 | 0.80 | K. N. 3 |
| 7 | 〃（2月15日） | ♂ | 4 | 1.10 | K. N. 4 |
| 8 | 飼育8ヶ月後死亡（3月25日生）飼育1ヶ月後採毛（4月25日） | ♀ | 1 | 0.50 | K. N. V—1 |
| 9 | 2ヶ月（5月25日） | ♀ | 2 | — | K. N. V—2 |
| 10 | 3ヶ月（6月25日） | ♀ | 3 | 0.90 | K. N. V—3 |
| 11 | 4ヶ月（7月25日） | ♀ | 4 | 1.20 | K. N. V—4 |
| 12 | 6ヶ月（9月25日） | ♀ | 6 | 1.50 | K. N. V—6 |
| 13 | 8ヶ月（11月25日） | ♀ | 8 | 2.50 | K. N. V—8 |
| 14 | 農家飼育動物から採毛（1月28日）（5月30日） | ♂ | 24以上 | 9.30 | N. C |
| 15 | 〃 | ♂ | 〃 | 7.80 | N. D |
| 16 | 〃 | ♀ | 〃 | 5.60 | N. E |
| 17 | 〃 | ♀ | 18以上 | 5.30 | N. F |

を進めることにした．そこで繊維の測定には剪毛かまたは抜き取った毛の一撮みを上毛，下毛の区別なく毛幹標準測定位の柄幅をマイクロメーターで検鏡測定し，下毛及び中間の小型毛は 1.8 $\mu$ 間隔で階級を取り，各級の度数及び葉幅，頸幅の平均値を求めた．

予備調査の結果上毛と下毛の混生する境域はほとんど例外なく柄幅 20 $\mu$ 内外の小型毛に含まれているので容易に調査を進めることが出来た．またこの小型毛の明葉毛以上の全上毛は柄幅を 10 $\mu$ 間隔の（級間 10 $\mu$ で）階級で頸幅，葉幅の平均値を調査した．さらに各毛を 5 mm 間隔で毛長の階級を分かって各部位を測定し，その毛長の級内で明葉毛と暗葉毛とを区分して測定値の平均値を求めた．

尚各毛種の Sump の標本，各種切片標本などの観察を交えて毛の分類を行った．

毛の発生と毛群の成立，3ケ月胎児，1腹の出産直後，月別幼獣，1，2，3，4ケ月目，及び，飼育幼獣8ケ月目の各種切片標本の観察を対照した．

毛皮の単位面積の毛包数と毛量，3ケ月胎児，各月別処分の幼獣皮の㊭切片，さらにそれ等のフオルマリン鞣革等により毛皮の単位面積 1 $cm^2$ 内の毛包，毛量を算出し，毛包の傾斜角を求めた．

換毛，換毛の状態は主として成獣皮の𝕽切片の連続切片，S 切片，L 切片で検鏡した．生毛から成獣の被毛まで，これは毛の発生調査と同じ各種幼獣皮の𝕽切片と採毛，飼育幼獣の月別採毛資料の調査を比較対照する．

主として毛群の成立の経過を調査した．

温度の影響，各月別に採った成獣皮の𝕽切片の調査と，冬季，夏季の採毛との比較調査等の対照によって求めた．

生体量，性別による変異，生体量を 7.5 kg（2 貫），5.5 kg（1.5 貫），3.5 kg（1 貫）内外に 3 区分して各区に♀♂各 5 頭分の採毛資料をあてこれを調査した．

## V 毛 の 分 類

1. 緒言，家畜には豚のように下毛がなく，上毛のみのもの，又緬羊のように下毛のみのものがある．改良を加えない土産種の豚や野猪では明らかに下毛を生じ，繊維を目的に改良された緬羊の merino 種も換毛前の生毛は上毛である．環境と人為によって形態と割合に変異はあるが，一般に多くの哺乳類の被毛は上毛と下毛から成立っている．

毛の呼称は従来各人各様で，一般には粗毛，綿毛と呼ばれるが，調査された動物でそれに最も適した特殊な表現を要する場合，或は習慣的に一般に使われるもの等多様である．

従来の文献から引用すると次のようである．

第 4 表 被毛 の 分 類

| over hair | | under hair | 一 般 呼 称 |
|---|---|---|---|
| bristle | awns | | |
| transitional hair | | | Botezat (1914) |
| Leithaar | Grannenhaar | Wollhaar | Toldt (1910) |
| | protective hair | | |
| guard hair<br>top hair | | fur, wool hair,<br>ground hair, under<br>wool, down, lanugo, | 一 般 呼 称 |
| 上 毛 | | 下 毛 | 阿 部(1) (1931) |
| 第 一 上 毛<br>上 毛 | 第 二 上 毛<br>中 毛 | | |
| 飛 毛（刺 毛）<br>とびげ，さしげ | 粗 毛（荒 毛）<br>そもう，あらげ | 棉 毛<br>わたげ，めんもう | 沢 山(36)<br>（毛皮一般）(1954) |
| 撒 硬 毛 | 硬 毛 | 綿 毛 | 上 坂(40)<br>（和牛）(1948) |

さらに Toldt は狐 (Canis Vulpes) 1935，の被毛を分類して (1) Leithaar, (2) Leitgrannenhaar, (3) dicke Grannenhaar, (4) dünne Grannenhaar, (5) Grannen-wollhaar, (6) Wollhaar として上毛を 3 区分し，上毛と下毛の中間に 1 区分粗綿毛（仮称）を入れている．又一部では中間型の毛を粗綿毛と呼ぶ習慣もある．筆者は Nutria の被毛の分類にあたって上毛，下毛の呼称をかりることにした．

2. 毛色の明暗と毛の分類との関係

(1) 毛の色調

F. W. Dry[11] (1926) によると，mouse や rat では毛の黒色素は常に皮質，髄質にあって

顆粒である．又黄色素は濃黄色の毛の部分では髄質の隔壁（実質）の上部に明らかに黄色の顆粒として存在しているが，その隔壁の基部では瀰漫性である．この黄色顆粒は常に平たい小球形であるとして globule 或は globular pigment と呼んでいる．又この小球は色素の発生と関係なく albino の場合にも髄質には無色として認められる（picric acid に染る）．この黄色と黒色顆粒とは髄質中に混在している．

明るい淡色の明帯（agouti band）では黄色は瀰漫性の色素であり，濃黄色の明帯では小球形の黄色素であるとし，いづれも髄質，皮質は透視出来る．

明帯の基部暗色部との境では顆粒性の黄色素の量は減じ，この黄色の小球の色調も淡色になる．又 Dry は Bateson 等が mouse で，black, chocolate, yellow, の色素顆粒をあげているのに対して，この chocolate の色素顆粒は彼の認めている黄色顆粒のことであろうと述べている．又石原[25]は褐色和牛でその基調色は淡色で黄色ないし黄褐色の色素顆粒を有するとしている．橋本[18]（1957）は野生色兎毛で淡色の色素顆粒を述べている．

Nutria の毛葉の明帯では淡黄色の瀰漫性色素，黄色顆粒，褐色顆粒，黒褐色顆粒，黒色顆粒の変異がある．又黄色顆粒の淡色ではほとんど無色に近く，それは Dry の場合のように，光線の乱反射の陰影によって認められる（写109，112図）．

又淡黄色の瀰漫性の基調色に黄色顆性が存在している．明帯の末端で毛芒との境の部分や基部の毛頸に接した部分では Dry の mouse の場合のように，黄色顆粒と黒褐色顆粒とが混在している，この黄色素顆粒の集積する明帯では Dry の mouse の場合と同様に透明である．

尚毛根部の髄質の痕跡の部分や全く髄質の欠けた部分でも中央に淡色の顆粒が認められる．又筆者は切片資料作製の際に，各種濃度のアルコール浸漬を経過してパラフィン包埋前のキシレン浸漬液中に生皮の切片を放置して約2ケ月後には毛の色素は完全に溶解して液は淡黄色に着色した．又芽毛，幼毛，青毛，或は成長直後の新毛の暗色部，とくに毛芒，毛頸部の黒色に近い色調は棍状毛として長期間生体に滞留する間に褪色して淡調になるようである．これは生皮の切皮の切片で観察する場合と棍状毛の場合とでは著しく相違することから明了である．

毛色はこの淡黄色の基調色に褐色，黒褐色顆粒が集積する時は帯黄褐色より鉄錆色の濃淡調を表わす．又半透明の凝膠色から乳濁色の基調色に褐色，黒褐色顆粒が集積するとき褐灰色，黒褐色，黒色を表わす．

(2) 上毛と下毛の分岐域

出生直後の生毛では大型毛の発育は遅れていて，幼毛であるが，小型毛にはすでに明葉毛と暗葉毛との青毛が混生している．生後1ケ月では生毛の多くは成熟期に近づいて小型暗葉毛も成長し，（換毛論で述べる）生毛の毛叢がほぼ完全に生え揃うことになる（成毛と青毛との混生毛叢）．

さらに生後約1ケ月から2ケ月までの1ケ月間に最も小型で繊細な暗葉毛の多くが二次的に発生して成長し，生毛の小型副側毛と共に代表的な大毛叢の基礎を形づくる．

以上のように生後約2ケ月では，生毛に2次副側毛の多くが追加されて Nutria の被毛はほぼ整ってくる．この毛叢の小型毛の明葉毛と暗葉毛から後継毛が生じて同じく次代の明葉毛，暗葉毛の混生毛叢を受け継ぐのである（写36図）．

生後4ケ月の Nutria の背部小型毛から，毛柄幅と毛葉幅との関係を調査した結果の1例

を示すと次のようである.

第 5 表　4 ケ月幼獣の小型毛の柄幅と葉幅の関係表

| No. | 柄幅(μ) 級間1.8μ, 代表値 | 暗葉毛 | | | | 明葉毛 | |
|---|---|---|---|---|---|---|---|
| | | 葉幅が柄幅と等しい場合 | | 最も頻度の高い場合 | | 最も頻度の高い場合 | |
| | | 葉幅 μ | % | 葉幅 μ | % | 葉幅 μ | % |
| 1 | 11.7 | 11.7 | 58 | 11.7 | 58 | 0 | 0 |
| 2 | 13.5 | 13.5 | 39 | 15.3 | 50 | 0 | 0 |
| 3 | 15.3 | 15.3 | 6 | 20.7 | 40 | 0 | 0 |
| 4 | 17.1 | | | 22.5 | 29 | 24.3 | 16 |
| 5 | 18.9 | | | 24.3 | 12 | 24.3 | 40 |
| 6 | 20.7 | | | 24.3 | 3 | 26.1 | 38 |
| 7 | 22.5 | | | | | 26.1 | 40 |

第 6 表　4 ケ月幼獣の小型毛の暗葉毛と明葉毛の比率表

| No. | 柄幅(μ) 級間 1.8 μ, 代表値 | 毛葉の明暗の比率 | |
|---|---|---|---|
| | | 暗葉毛 % | 明葉毛 % |
| 1 | 9.9 | 100 | 0 |
| 2 | 11.7 | 100 | 0 |
| 3 | 13.5 | 100 | 0 |
| 4 | 15.3 | 100 | 0 |
| 5 | 17.1 | 64 | 36 |
| 6 | 18.9 | 29 | 71 |
| 7 | 20.7 | 8 | 92 |
| 8 | 22.5 | 0 | 100 |

暗葉が100%の小型毛でその柄幅が 9.9 μ, 11.7 μ, 13.5 μ, 15.3 μ, の場合は葉幅と柄幅とが等しく, 先き細りで, 縫針状のものが各区に58%, 39%, 6%と減少しており, 明葉毛を含む柄幅 17.1 μ 以上では葉幅は凡て柄幅より広い. 以上のように小型毛では $\frac{葉幅}{柄幅} \geqq 1$ であるが, 明葉毛と暗葉毛の混生域にいくと $\frac{葉幅}{柄幅} > 1$ に変化する. 尚混生域では暗葉毛の数は階級毎に減少して明葉毛と交代している. この間において暗葉毛の葉幅（平均値）は常に明葉毛より小であるが, 混生域では暗葉幅, 明葉幅の増加率はほぼ同一で平行している. この域を出て明葉毛のみとなれば急角度に上昇している. これを直線に直して表わすと暗葉幅と, 明葉幅の傾向は一層明白である（第 8 図).

生後 1 ケ月より 4 ケ月と換毛につれて明暗葉毛の混生域が拡長して来ると共に（第 9, 10, 11, 12図), 前月までは明葉毛のみで高角度を保っていた階級が混生域に加わると明葉幅は減少して暗葉幅に迫って平行している（第14, 15, 16, 17図). 尚この間, 暗葉毛の混生域では暗葉毛と小型明葉毛との毛葉の断面は正円に近く（写36図), 毛小皮は擦り目の細い縄紋型に並んだ冠状で毛葉は彎曲している（写35, 25図).

又髄質は中心から外れて彎曲内面に偏り暗葉の皮質は褐色顆粒多く, 黒褐色であるが, 明葉は淡黄色の透明である（写24, 34図).

尚毛柄の毛小皮は単純冠状で, 断面は正円に近い. 又外部の形態と色調を比較すると,

暗葉毛では毛体の後半は縫針状に先き細りで濃褐色である．この混生域の明葉毛は一般に毛葉の先端，毛芒に接近して透明帯が偏り，やや葉幅を増すとともに毛葉の前後（毛芒，毛頸に連続して）に黒褐色部を残して中央部が色調が淡色になり均一な黄色透明な基調色に褐色素顆粒の群がる斑紋が明了に浮き出して，明度M　2Mの明葉毛を表わしている．

この域を脱して小型第2上毛の級に入ると長径，短径（a，b）の差を生じて断面は楕円形になり明帯の最広幅部から褐色素顆粒は消失して，黄色顆粒が微かに視野に浮き出して均一な明色に変り（淡黄色→帯赤黄色，腹毛は乳濁色半透明から淡黄乳濁色である）毛葉は濃褐色の毛頸部と黒褐色の毛芒部に挟まれた幅の広い透明帯を表わす．これを明度3Mとする（写41，42，43図）．この明，暗葉毛の混生毛叢を上毛と下毛の分岐域とみて暗葉毛を下毛の終点とし明葉毛を上毛の起点とする．作図（第9，10，11，12図）は凡て明，暗葉毛の分岐域を百分率度数折線で示している．又生後4ケ月の毛叢から総毛の度数折線の例をとって明暗葉毛の混生比率に従って両分し上毛，下毛の2つの度数折線を示した（第16表，第13図）．

Nutria の被毛では上述のように上毛と下毛の分岐する境界を明らかに認めえたので，上毛と下毛の中間型で両区分に股がっているという意味から発した Woll-grannenhaar（所謂る粗綿毛）ではなく分岐域のもの，それから分離した上毛，下毛というので，この混生域の小型毛を分岐毛（cross hair）としその明葉毛を分岐上毛（cross overhair），暗葉毛を分岐下毛（cross underhair）と名づけることにする．尚この分岐域外の最小暗葉毛を単に下毛（underhair）と呼ぶ．

下毛は小型下毛の二次的な発生で生後3ケ月頃から定形的な落ち着きを見せているが，生後8ケ月頃からやや幅を増して，約1ケ年で成毛を完成するように見受けられる（換毛論で述べる）．

下毛或は下毛＋分岐下毛の場合のいづれも度数分布の最頻数は極端に左方に偏していて資料の増加によって更にその特殊性を増すのみで正規分布ではない（第21表第49→56図）．

生後8ケ月（K. N. V-8）の背毛の下毛＋分岐下毛の度数分布を調査表のように算出して鑑別規準（criterion）K＝−0.2585 で，K<O であるからピアソン系の曲線では第一型に属している（第21，22，23表）．成獣の下毛及び分岐下毛の柄幅の平均値は調査表（第75表，97，98表）のように背毛で 12→16 $\mu$，腹毛で 10→12 $\mu$ の間にある．

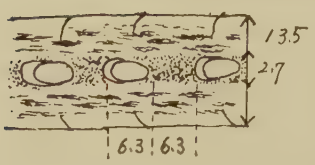
背下毛の観察例を示せば次のようである．芒毛の尖端は2 $\mu$，尖端の透明部は約 0.14 mm で黒褐色素顆粒が点在していて髄質はない，その基部の幅は 4.5 $\mu$，この基部から黒褐色素顆粒の多い毛芒に入り 0.4 mm 続いてその基部は幅 13.5 $\mu$ である．毛葉は毛芒の尖端より全毛長の$\frac{1}{4}$即ち5 mm 付近が最広部で葉幅 15.3 $\mu$，髄幅 2.7 $\mu$ で黒褐色顆粒が皮質に多い．

毛頸は全毛長の略中央で頸幅 11.7 $\mu$，毛柄は毛球下端より全毛長の$\frac{1}{4}$付近で柄幅 13.5 $\mu$，髄幅 2.7 $\mu$，毛球下端より 2.5 mm の毛根上位で髄質の痕跡の始まる付近は幅 11.7 $\mu$，毛根は下端より 1.5 mm で根幅は 9.9 $\mu$，棍毛球の長さ 0.177 mm で球幅 15.3 $\mu$ である．

写真（写26図）と対照すれば明瞭であるが，尚お細部の観察については皮質，髄質，色素の関係で述べている．

分岐上毛の観察例，毛長 2.5 cm 明度2Mの明葉毛芒，先は 0.25 mm 透明で毛芒の長

第5図 毛 柄 略 図




→毛の先端に向って

| | |
|---|---|
| 柄 幅 | 13.5 μ |
| 髄 幅 | 2.7 μ |
| 気 室 | 6.3 μ |
| 隔壁(実質) | 6.3 μ |

さは約3.5 mmで，その基幅は15.3 μ，側幅は9.9 μ，明帯の最広部は毛芒先から0.56 mmにあって，葉幅は22.5 μ，髄幅 8.1 μ，明帯の長さは 2.5 mm で，この葉幅の最広部は明帯の基部に近い．即ち明度2 Mの規格に当る．

毛頸部は毛の中央を占め頸幅は両端から 1.25 cm 付近で，頸幅 17.1 μ，髄幅 6.3 μ，毛柄幅はは毛の下端より 0.6 cm 付近で，22.5 μ 髄幅 11.7 μ，根幅は毛球端より 0.7 mm で 13.5 μ，棍毛球の長さは 0.25 mm で，球幅は 15.3 μ である．

(3), 上毛の変異

分岐域を離れた小型明葉毛より暗葉の長大毛までその代表的なものを例にとって示せば次のようである．

1) 暗葉毛，毛長 6.5 cm

毛芒の先端 6.3 μ，無色又は黒色顆粒点在の半透明の部分が 0.7 mm つづき，その基部の幅 13.5 μ，これより毛芒の濃色部に入り，黒褐色部が約 2.5 mm つづく，この色素のつきる辺りに髄質の痕跡が現われる．ここを毛芒の基部とする．この基幅 80.1 μ，側幅 51.3 μ である．

毛芒の先端より約1 cm で毛葉の広幅部で葉幅 (a) は 164.7 μ，側幅 (b) は 62.6 μ，髄幅 (c) 101.7 μ，この毛葉部は鉄錆色の暗葉を示している．毛頸部に下り，頸幅は125.1 μ，側幅 56.7 μ，髄幅 80.1 μ，でやや淡色になる．棍毛球は約 0.5 mm で球幅は 96.3 μ，毛根部は棍球端から 0.7 mm—1 mm の位置で根幅 65.7 μ (100%)，側幅 53.1 μ (80%) で，いづれも色素顆粒なく乳濁色である．

註，毛芒とは毛の尖端の髄質の痕跡までをいう．芒先とは毛芒の先端の透明部をいう．

2) 明葉毛，毛長 4.5 cm，明度 3 M,

毛芒の先端透明の部分 0.35 mm，その基幅 11.7 μ，これより毛芒の濃色部に入り，黒褐色部が約 2.84 mm つづき，明帯との境で半透明となり髄質の痕跡を認める．この部分の幅は 51.3 μ (100%) で側幅は 31.5 μ (60%) である．毛芒の尖端から約 7.1 mm が毛葉の広幅部で葉幅は 184.5 μ，側幅 74.7 μ．(40%), 髄幅 146.7 μ である．この広幅部は中心に左右に長さ約 8 mm の明かるい透明の黄色の明帯がある．

毛頸部では幅は狭くなり，毛幹のほぼ中央で最も狭い部分の頸幅は 94.5 μ (100%)，側幅 62.1 μ (65%)．髄幅 40.5 μ で黒褐色である．

毛柄部では柄幅は 121.5 μ (100%)，側幅 53.1 μ (44%)，髄幅 78.3 μ で，やや淡色になり灰褐色である．

棍球の長さは 0.7 mm で中央の球幅は 67.5 μ，棍球端から約1 mm の部分が毛根の最狭部で，根幅 62.1 μ，側幅 49.5 μ である．

3) 小型明葉毛，毛長 3.0 cm 明度 3 M,

毛芒尖端即ち芒先の透明部 0.5 mm その基幅 13.5 μ，毛芒の長さ約 3.0 mm その基

幅 29.7 μ，側幅 17.1 μ である．これより明帯が約 6.0 mm つづき，芒先より約 6.0 mm が中央の広幅部で葉幅は，150.3 μ（100%），側幅 60.3 μ（40%），骸幅 114.3 μ，毛頸は毛幹の中央で最狭部を示す頸幅は 62.1 μ，側幅は 44.1 μ，髄幅 26.1 μ である．毛幹部は 98.1 μ，側幅 44.1 μ，髄幅 62.1 μ である．

棍球の長さは 0.5 mm で，球幅は 71.1 μ，棍球の端より 0.71 mm．で髄質の痕跡と棍球のほぼ中間に当るところが毛根の最狭部で，根幅 53.1 μ（100%），側幅 42.3 μ（80%），髄質の痕跡は毛球端より約 1 mm で始まる．

以上の観察と顕微鏡写真図との対照により背部上毛の外形は次のように総括される．

背部上毛，棍球，毛根の乳濁色から半透明の凝膠色の毛柄を経て，毛葉では半透明の淡黄色の基調色である．この棍球を含む毛根までは色素顆粒を欠いて無地であるが，これより褐色素顆粒が中心に点在して，ついに色素顆粒が髄質の痕跡に集り，これを中心とする皮質に顆粒が群在して褐色となり髄幅が増して，毛柄では皮質とそれに沿って附着する髄質に色素顆粒が多く集結しているので，髄質を中心にその周辺は灰色，灰褐色である．

毛柄をさかのぼって毛頸では褐色素顆粒の集積が多量になって黒褐色から黒色となる．

毛葉では急に黒褐色素を減じて，淡黄色の基調色に褐色，黄色の混合帯を生じ漸次褐色素を失い黄色顆粒が集積して透明の明帯を表わす．この明帯は黄色顆粒の増加により淡黄色から黄金色を示す．

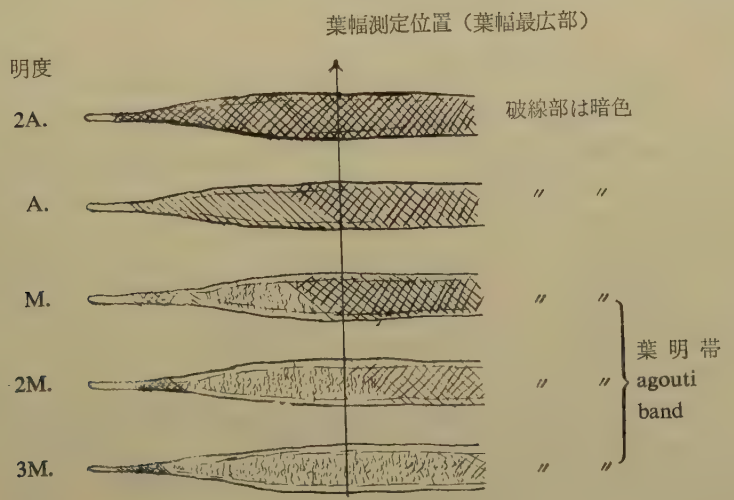
毛葉から毛芒に移るに従って黒褐色素顆粒が増して毛芒は黒褐色から黒色に変じて，その毛芒の尖端即ち芒先は半透明→透明の凝膠色の無色か又は黒褐色顆粒が点在している．

一般に明帯をはさむ毛芒と毛頸は濃色（黒褐色又は黒色）で毛柄は淡色（灰色）である．この明帯は第 2 上毛ではその毛葉の大部分を占めて明度 2 M— 3 Mであるが，大型上毛の第 1 上毛では明帯は幅を減じて毛葉の先端に偏り明度は 2 M—Mとなり，遂には明帯を失って帯黄黒褐色の明度 A から黒褐色，黒色の明度 2 A の不透明な暗葉毛となる．

この明度を図示せば次のようである（第 6 図）．

更に毛長及び柄幅の区分から明度の比較により上毛の変異は一層明らかになってゆき，

第　6　図



TOLDT (1935) が狐の被毛で示している Leit-haar, Leit-grannenhaar, dicke Grannenhaar, dünne Grannenhaar, Grannen-wollhaar, Woll-haar, に準じた分類がえられる.

折線図（第22—25図）の示すように毛長，毛幅，色調の間にはやや明らかな相関関係が認められる．上毛と下毛の分岐域では上毛はおおむね明帯の明度は 2 M級であるが，この域を離れて，毛長，柄幅が増すと明度 3 Mとなる（2.5—3.0 cm dünne Grannen-haar. 更に毛長が増すと（3.0—5.0 cm）毛葉の最も広い部分が前方に移る傾向があり，明帯は 3 M—2 Mを示して毛の中央に当る毛頸のくびれが深く，全被毛中で柄幅，葉幅とも最大の場合が多い．この外形は古代の矛（ほこ）のようで，代表的な第 2 上毛である（dicke Grannenhaar).

毛長が更に増すと（5—8 cm)，柄幅，葉幅はやや減少し頸幅は増して，毛頸のくびれが浅いか又はくびれが全く無くなり，ついに柄幅より広くなって，毛葉は先き太りで，外形は古代の太刀（長剣）に似ている.

色調は全毛を通じて暗色であるが，とくに毛頸のくびれが浅くなるほど毛葉部の明帯が短く，毛芒に近く偏っているので，毛葉は暗色を増してゆき遂に全毛が暗褐色，黒褐色に変り（M→A→2 A）代表的な第 1 上毛となる.

これは第 2 上毛と第 1 上毛の中間型から第 1 上毛の変異を表わしている（Leit-grannenhaar→Leithaar).

次に腹部上毛の例を示すと.

腹部上毛の例，明葉毛，毛長 3.8 cm，毛芒 2.1 mm これは芒先から髄質の痕跡までを原則とする，この間に褐色顆粒なし，毛芒の基幅 63.9 μ，毛葉の中央は芒先から約 1.26 cm が最広部で，葉幅 150.3 μ（100%)，側幅 60.3 μ（40%)，髄幅 78.3 μ，淡黄色透明である．毛頸の最狭部は毛幹の略中央で芒先から約 2 cm にあり，頸幅 81.9 μ，側幅 35.1 μ，髄幅 27.1 μ，灰色である．毛柄は棍球端から約 1 cm の柄幅は 89.1 μ，側幅は 51.3 μ，髄幅 56.7 μ で，淡灰色である．毛根は棍球端をら 0.71 mm が最狭部で，根幅は 49.5 μ，側幅は 35.1 μ，乳濁色の半透明である．根球の長さは 0.35 mm で，球幅は 62.1 μ，乳濁色の半透明である．棍球の長さは 0.35 mm で，球幅は 62.1 μ，乳濁色の半透明である．髄質の痕跡は棍球端から約 1.0 mm で始まっている．腹部上毛の観察を総括すると次のようである.

毛葉，棍球がやや乳濁色を帯びているが全毛幹が略半透明の凝膠色である．毛芒，毛葉とも色素顆粒を欠いて単に薄い乳濁色の透明で中心に髄質の陰影を表わしているもの，毛葉が髄質を中心に周辺の皮質に黄色顆粒が集結して中央が淡黄色から黄金色の色調を表わすもの等がある．分岐上毛及び小型上毛は全幹が薄墨色（淡灰色）で毛葉に乳白色又は帯黄色の明帯を表わしている．尚個体によっては明らかな明帯がなく毛柄，毛頸を経て，黒褐色顆粒が髄質，皮質に均一に分散していて，毛葉では黄色顆粒と混在して半透明の場合もある．小型上毛の数は背毛に比較して少い.

腹部上毛はがいして変異に乏しく毛脚の揃った第 2 上毛のみで，明らかに第 1 上毛の性格を備えた上毛を欠いでいる.

尚腹部上毛の毛葉の皮質は背毛に比較して分厚いようである．毛柄の断面は小型上毛では正円に近く，大型上毛は一般に長径，短径の差の少い楕円形を示して，髄質は拡大して背毛に比べて空虚の度は高いようである（写114図).

## 第7表　動物記号K.N.4背毛，下毛と上毛の分岐域の算出基礎表

（柄幅と葉幅の相関表）　暗葉毛はA．明暗混毛中の明葉毛はM．暗葉毛，数字は太字

| 葉幅級間 1.8 代表値 | | 10.8～12.6 | 12.6～14.4 | 14.4～16.2 | 16.2～18.0 | 18.0～19.8 | | 19.8～21.6 | | 21.6～23.4 | | 23.4～25.2 | | 25.2～27.0 | | 27.0～28.8 | 28.8～30.6 | 30.6～32.4 | 32.4～34.2 | 34.2～36.0 | 36.0～37.8 | 暗葉数 | 明葉数 | 合計 | % 暗葉 | % 明葉 | 明暗葉幅平均 μ | 暗葉幅平均 μ | 明葉幅平均 μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 明暗 / 柄幅級間 1.8 | 代表値 μ | 11.7 | 13.5 | 15.3 | 17.1 | 18.9 | | 20.7 | | 22.5 | | 24.3 | | 26.1 | | 27.9 | 29.7 | 31.5 | 33.3 | 35.1 | 36.9 | | | | | | | | |
| | | A | A | A | A | A | M | A | M | A | M | A | M | A | M | M | M | M | M | M | M | | | | | | | | |
| 10.8～12.6 | 11.7 | %58 | 42 | | | | | | | | | | | | | | | | | | | 96 | 0 | %100 | 100 | 0 | 12.45 | 12.45 | |
| | | 56 | 40 | | | | | | | | | | | | | | | | | | | | | 96 | | | | | |
| 12.6～14.4 | 13.5 | | %39 | 50 | 7 | 4 | | | | | | | | | | | | | | | | 84 | 0 | %100 | 100 | 0 | 14.85 | 14.85 | |
| | | | 33 | 42 | 6 | 3 | | | | | | | | | | | | | | | | | | 84 | | | | | |
| 14.4～16.2 | 15.3 | | | % 6 | 12 | 28 | | 40 | | 14 | | | | | | | | | | | | 43 | 0 | %100 | 100 | 0 | 19.65 | 19.65 | |
| | | | | 3 | 5 | 12 | | 17 | | 6 | | | | | | | | | | | | | | 43 | | | | | |
| 16.2～18.0 | 17.1 | | | | | %10 | 3 | 22 | 3 | 29 | 11 | 3 | 16 | 1 | 3 | | | | | | | 47 | 26 | %100 | +64 | −36 | 22.11 | 21.50 | 23.19 |
| | | | | | | **7** | 2 | **16** | 2 | **21** | 8 | **2** | 12 | **1** | 2 | | | | | | | | | 73 | | | | | |
| 18.0～19.8 | 18.9 | | | | | | | %2 | | 10 | 15 | 12 | 40 | 4 | 15 | 2 | | | | | | 19 | 48 | %100 | −29 | +71 | 24.19 | 23.73 | 24.38 |
| | | | | | | | | **1** | | **7** | 10 | **8** | 27 | **3** | 10 | 1 | | | | | | | | 67 | | | | | |
| 19.8～21.6 | 20.7 | | | | | | | | | %2 | 8 | 30 | | 38 | | 13 | 10 | | | | | 3 | 37 | %100 | −8 | +92 | 25.92 | 24.30 | 26.05 |
| | | | | | | | | | | 1 | **3** | 12 | | 15 | | 5 | 4 | | | | | | | 40 | | | | | |
| 21.6～23.4 | 22.5 | | | | | | | | | | | | | %40 | | 13 | 20 | 7 | | 13 | 7 | 0 | 30 | %100 | 0 | 100 | 29.34 | | 29.34 |
| | | | | | | | | | | | | | | 12 | | 4 | 6 | 2 | 0 | 4 | 2 | | | 30 | | | | | |
| | 柄幅平均 μ | 11.7 | 12.51 | 13.62 | 14.32 | 15.75 | | 16.30 | | 17.54 | | 18.92 | | 20.40 | | 21.24 | 21.78 | 22.50 | 0 | 22.5 | 22.5 | | | | | | | | |

第8表　動物記号 K. N. 4 背毛，小型毛の毛柄幅と毛葉幅の相関表（a は暗葉毛　m は明葉毛）

| 毛葉の明暗→ | | 葉幅代表値 μ → | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 9.9 | 11.7 | 13.5 | 15.3 | 17.1 | 18.9 | | 20.7 | | 22.5 | | 24.3 | | 26.1 | | 27.9 | 29.7 | 31.5 | 33.3 | 35.1 | 36.9 | 38.7 | 40.5 | 42.3 | 44.1 | 45.9 | 47.7 | 49.5 | 51.3 | 53.1 | 54.9 | 56.7 | 58.5 | 60.3 |
| | | a | a | a | a | a | a | m | a | m | a | m | a | m | a | m | a | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m | m |
| 柄幅代表値 μ ↓ | 9.9 | 32 | 24 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 11.7 | | 21 | 16 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 13.5 | | | 17 | 15 | 4 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 15.3 | | | | 3 | 6 | 14 | | 19 | | 6 | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 17.1 | | | | | | 9 | 2 | 22 | 2 | 24 | 10 | 6 | 13 | 1 | 3 | | | | | | | | | | | | | | | | | | | | | | |
| | 18.9 | | | | | | | | 1 | | 18 | 11 | 12 | 33 | 3 | 10 | 1 | 3 | | | | | | | | | | | | | | | | | | | | |
| | 20.7 | | | | | | | | | | | 1 | 8 | 16 | 1 | 23 | | 11 | 6 | 1 | | | | | | | | | | | | | | | | | | |
| | 22.5 | | | | | | | | | | | | | 1 | | 16 | | 8 | 9 | 2 | 1 | 4 | 2 | | | | | | | | | | | | | | | |
| | 24.3 | | | | | | | | | | | | | | | 2 | | 9 | 1 | 2 | 2 | 3 | 1 | | 1 | | | | | 1 | | 2 | | | | | | |
| | 26.1 | | | | | | | | | | | | | | | | | 4 | 0 | 1 | 2 | 1 | 3 | 1 | 1 | 1 | | | | | | | 1 | | | | | |
| | 27.9 | | | | | | | | | | | | | | | | | | | 3 | 2 | 3 | 2 | 1 | 0 | 1 | 0 | 2 | 1 | 1 | | | | | | | | |
| | 29.7 | | | | | | | | | | | | | | | | | 1 | | | 1 | 1 | 1 | | 1 | | 1 | 1 | | | 1 | | 1 | | | | | |
| | 31.5 | | | | | | | | | | | | | | | | | | | 2 | 0 | 2 | 2 | | | 1 | 0 | 1 | | 1 | 1 | | 1 | | | | | |
| | 33.3 | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | 1 | | | | | | 2 |
| | 35.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | |
| | | 32 | 45 | 33 | 18 | 10 | 24 | | 42 | | 48 | | 27 | | 5 | | 1 | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | 2 | | 2 | | 22 | | 63 | | 54 | | 35 | 16 | 11 | 8 | 14 | 11 | 2 | 3 | 3 | 2 | 4 | 1 | 4 | 1 | 5 | 1 | 2 | 0 | 2 | | |
| | 柄幅平均 μ → | | | | | | | 17.1 | | 17.1 | | 18.2 | | 19.0 | | 20.8 | | 23.3 | 21.9 | 26.1 | 26.1 | 26.1 | 26.9 | 27.0 | 26.7 | 28.5 | 31.5 | 29.3 | 27.9 | 29.7 | 31.5 | 29.3 | 26.1 | 30.6 | 0 | 33.3 | | |

| 毛葉の 明 暗→ | 葉幅代表値 μ → 62.1 m | 63.9 m | 65.7 m | 67.5 m | 69.3 m | 71.1 m | 72.9 m | 74.7 m | 暗葉毛 a | 明葉毛 m | 合計 a+m | 分岐率 % a | m | 暗葉幅平均 μ | 明葉幅平均 μ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 柄幅代表値 μ ↓ 9.9 | | | | | | | | | 56 | | 56 | 100 | | 10.67 | | |
| 11.7 | | | | | | | | | 37 | | 37 | 100 | | 12.48 | | |
| 13.5 | | | | | | | | | 37 | | 37 | 100 | | 14.76 | | |
| 15.3 | | | | | | | | | 49 | | 49 | 100 | | 19.71 | | |
| 17.1 | | | | | | | | | 62 | 30 | 92 | 67.4− | 32.6+ | 21.59 | 23.28 | 分岐域 |
| 18.9 | | | | | | | | | 35 | 57 | 92 | 38.0 | 62.0 | 23.53 | 24.46 | |
| 20.7 | | | | | | | | | 9 | 58 | 67 | 13.4+ | 86.5− | 24.50 | 26.35 | |
| 22.5 | | | | | | | | | | 43 | 43 | | 100 | | 28.90 | |
| 24.3 | | | | | | | | | | 24 | 24 | | 100 | | 33.38 | |
| 26.1 | | | | | | | | | | 15 | 15 | | 100 | | 35.50 | |
| 27.9 | 1 | | | | | | | | | 17 | 17 | | 100 | | 39.30 | |
| 29.7 | | | | | | | | | | 9 | 9 | | 100 | | 41.50 | |
| 31.5 | | | | | | 2 | | 1 | | 14 | 14 | | 100 | | 47.80 | |
| 33.3 | 1 | | 1 | | | | | | | 6 | 6 | | 100 | | 57.60 | |
| 35.1 | | | | | | 1 | | | | 3 | 3 | | 100 | | 62.40 | |
| | 2 | 0 | 1 | 0 | 0 | 3 | 0 | 1 | | | | | | | | |
| | 30.6 | 0 | 33.3 | 0 | 0 | 32.7 | 0 | 31.5 | | | | | | | | |

上表において，分岐毛を含む小型毛の葉幅と柄幅との関係式は直線の方程式 $Y=a+bx$ として，最小自乗法による次の規準方程式で解く，

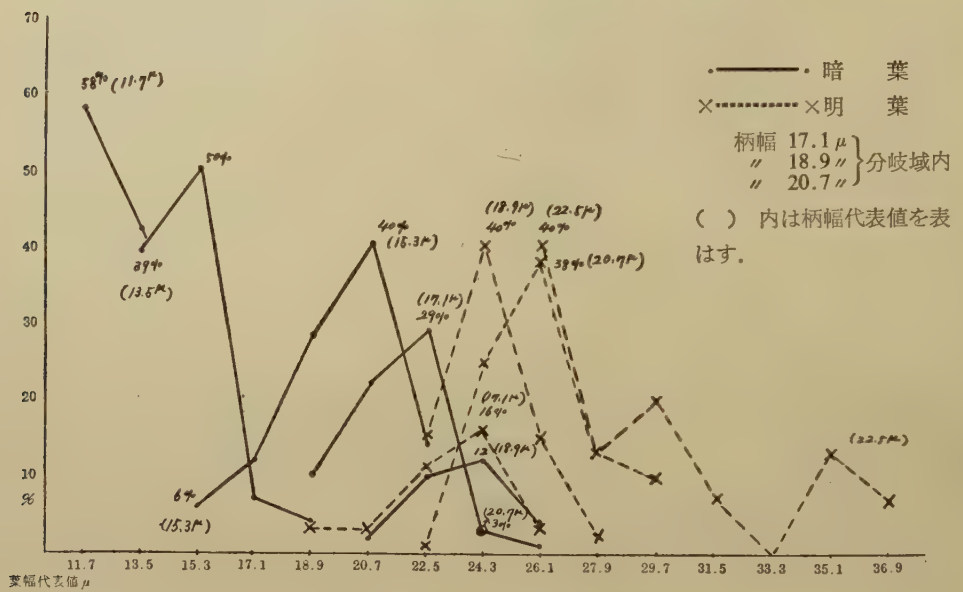
$$S(n)\,a+S(x)\,b=S(y)\cdots\cdots(1)$$

$$S(x)\,a+S(x^2)\,b=S(xy)\cdots\cdots(2)$$

a, b を求めて，$Y=a+bx$ に代入し期待値をえた．

以上のように腹毛の毛芒，毛葉は透明で光沢に富み，毛柄も淡灰色の半透明であり，外観は光沢の強い淡黄灰色→淡黄褐色である．

第 7 図　小型毛の毛柄と毛葉との関係図 (K. N. 4 背毛)




第 9 表　暗 葉 毛 の 場 合　(作図のA線)

Animal no. K. N. 4背毛

| 柄幅, 級間 1.8μ | | | 観察値 | x | n | $x^2$ | xy | 期待値 |
|---|---|---|---|---|---|---|---|---|
| μ | μ | 代表値 μ | y | | | | | Y μ |
| 9.0 | 10.8 | 9.9 | 10.7 | 0 | 1 | 0 | 0 | 10.66 |
| 10.8 | 12.6 | 11.7 | 12.5 | 1 | 1 | 1 | 12.5 | 13.17 |
| 12.6 | 14.4 | 13.5 | 14.8 | 2 | 1 | 4 | 29.6 | 15.68 |
| 14.4 | 16.2 | 15.3 | 19.7 | 3 | 1 | 9 | 59.1 | 18.19 |
| 16.2 | 18.0 | 17.1 | 21.6 | 4 | 1 | 16 | 86.4 | 20.70 |
| 18.0 | 19.8 | 18.9 | 23.5 | 5 | 1 | 25 | 117.5 | 23.21 |
| 19.8 | 21.6 | 20.7 | 24.5 | 6 | 1 | 36 | 147.0 | 25.72 |
| | | | 127.3 | 21 | 7 | 91 | 452.1 | |

$7a+21b=127.3\cdots(1)$　　$a=10.66\,\mu$　　$Y=bx+a$

$21a+91b=452.1\cdots(2)$　　$b=2.51\,\mu$　　$=2.51x+10.66$

$x=0\cdots Y=10.66\,\mu$

$x=6\cdots Y=25.72\,\mu$

第10表　分岐上毛＋小型第2上毛の場合（作図のB線）

Animal no. K.N.4 背毛

| 柄幅，級間 1.8μ | | | 観察値 | x | n | $x^2$ | xy | 期待値 |
|---|---|---|---|---|---|---|---|---|
| μ | μ | 代表値μ | y | | | | | Y μ |
| 16.2 | 18.0 | 17.1 | 23.3 | 0 | 1 | 0 | 0 | 18.99 |
| 18.0 | 19.8 | 18.9 | 24.5 | 1 | 1 | 1 | 24.5 | 22.84 |
| 19.8 | 21.6 | 20.7 | 26.4 | 2 | 1 | 4 | 52.8 | 26.69 |
| 21.6 | 23.4 | 22.5 | 28.9 | 3 | 1 | 9 | 86.7 | 30.54 |
| 23.4 | 25.2 | 24.3 | 33.4 | 4 | 1 | 16 | 133.6 | 34.39 |
| 25.2 | 27.0 | 26.1 | 35.5 | 5 | 1 | 25 | 177.5 | 38.24 |
| 27.0 | 28.8 | 27.9 | 39.3 | 6 | 1 | 36 | 235.8 | 42.09 |
| 28.8 | 30.6 | 29.7 | 41.5 | 7 | 1 | 49 | 290.5 | 45.94 |
| 30.6 | 32.4 | 31.5 | 47.8 | 8 | 1 | 64 | 382.4 | 49.79 |
| 32.4 | 34.2 | 33.3 | 57.6 | 9 | 1 | 81 | 518.4 | 53.64 |
| 34.2 | 36.0 | 35.1 | 62.4 | 10 | 1 | 100 | 624.0 | 57.49 |
| | | | 420.6 | 55 | 11 | 385 | 2526.1 | |

11a＋ 55b＝ 420.6…(1)　　a＝18.99　　Y＝bx＋a

55a＋385b＝2526.2…(2)　　b＝3.85　　＝3.85x＋18.99

x＝ 0…… Y＝18.99μ

x＝10…… Y＝57.49μ

第11表　分岐域を離れた小型第2上毛の場合（作図のC線）

Animal no. KN.4 背毛

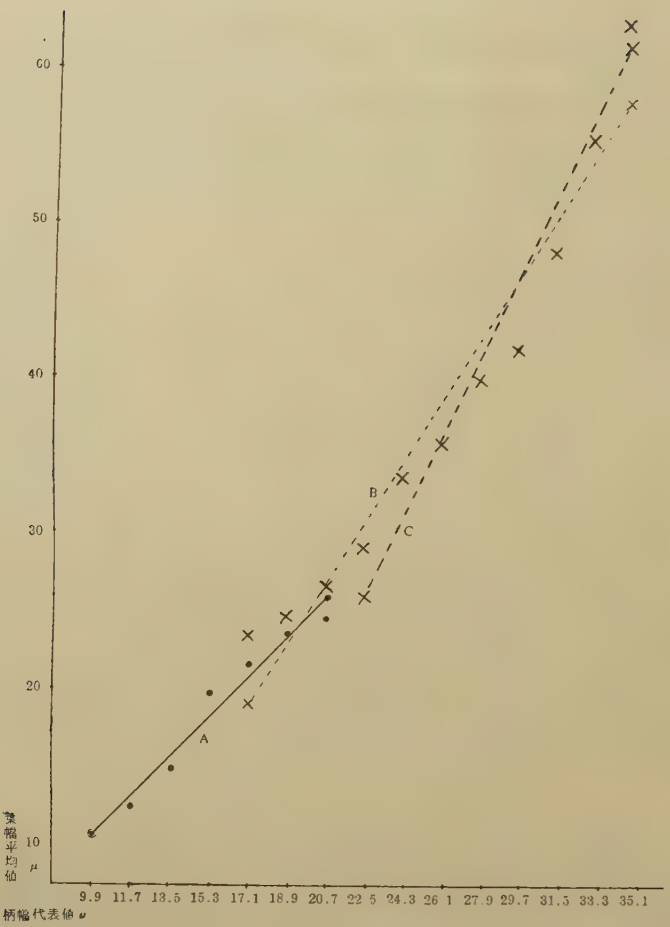
| 柄幅，級間 1.8μ | | | 観察値 | x | n | $x^2$ | xy | 期待値 |
|---|---|---|---|---|---|---|---|---|
| μ | μ | 代表値μ | y | | | | | Y μ |
| 21.6 | 23.4 | 22.5 | 28.9 | 0 | 1 | 0 | 0 | 25.77 |
| 23.4 | 25.2 | 24.3 | 33.4 | 1 | 1 | 1 | 33.4 | 30.78 |
| 25.2 | 27.0 | 26.1 | 35.5 | 2 | 1 | 4 | 71.0 | 35.79 |
| 27.0 | 28.8 | 27.9 | 39.3 | 3 | 1 | 6 | 117.9 | 40.80 |
| 28.8 | 30.6 | 29.7 | 41.5 | 4 | 1 | 16 | 166.0 | 45.81 |
| 30.6 | 32.4 | 31.5 | 47.8 | 5 | 1 | 25 | 239.0 | 50.82 |
| 32.4 | 34.2 | 33.3 | 57.6 | 6 | 1 | 36 | 345.6 | 55.83 |
| 34.2 | 36.0 | 35.1 | 62.4 | 7 | 1 | 49 | 436.8 | 60.84 |
| | | | 346.4 | 28 | 8 | 137 | 1409.7 | |

8a＋ 28b＝ 346.4…(1)　　a＝25.77 μ　　Y＝bx＋a

28a＋137b＝1409.7…(2)　　b＝ 5.01 μ　　＝ 5.01x＋25.77

x＝0……Y＝25.77 μ

x＝7……Y＝60.84 μ




第 8 図　小型毛の毛柄と毛葉との関係図 (2) K. N. 4 背毛 (分岐域を含む)

第 12 表　下毛と上毛の分岐域 (この様式の調査表より第 9～12 図をえた)

動物記号　K. N. 1 背毛 Dorsal Hair

| 毛柄幅 (μ) | | | 暗葉幅 (μ) | | | | 明葉幅 (μ) | | | | 暗葉，明葉，幅平均 | | | | 明暗比率 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 8 μ 間隔階級 | | 代表値 | 最小 | 最大 | 平均値 | 平均数 | 最小 | 最大 | 平均値 | 平均数 | 最小 | 最大 | 平均値 | 平均数 | 暗葉 % | 明葉 % |
| 5.4 | 7.2 | 6.3 | | | | | | | | | | | | | | |
| 7.2 | 9.0 | 8.1 | | | | | | | | | | | | | | |
| 9.0 | 10.8 | 9.9 | | | | | | | | | | | | | | |
| 10.8 | 12.6 | 11.7 | 11.7 | 15.3 | 13.86 | 105 | | | | | 11.7 | 15.3 | 13.86 | 105 | 100 | 0 |
| 12.6 | 14.4 | 13.5 | 15.3 | 20.7 | 17.33 | 96 | | | | | 15.3 | 20.7 | 17.33 | 96 | 100 | 0 |
| 14.4 | 16.2 | 15.3 | 17.1 | 20.7 | 19.30 | 54 | | | | | 17.1 | 20.7 | 19.30 | 54 | 100 | 0 |
| 16.2 | 18.0 | 17.1 | 17.1 | 27.9 | 21.65 | 17 | | | | | 17.1 | 27.9 | 21.65 | 17 | 100 | 0 |
| 18.0 | 19.8 | 18.9 | 20.7 | 29.7 | 23.85 | 8 | — | 20.1 | 26.10 | 3 | 20.7 | 26.1 | 24.66 | 11 | 73− | 27+ |
| 19.8 | 21.6 | 20.7 | 26.1 | 33.3 | 29.10 | 3 | 27.9 | 45.9 | 36.18 | 7 | 26.1 | 45.9 | 34.02 | 10 | 30 | 70 |
| 21.6 | 23.4 | 22.5 | — | 31.5 | 31.50 | 1 | 27.9 | 54.9 | 38.93 | 8 | 31.5 | 54.9 | 38.10 | 9 | 11+ | 89− |
| 23.4 | 25.2 | 24.3 | | | | | | | | | | | | | | |
| 25.2 | 27.0 | 26.1 | | | | | | | | | | | | | | |
| 27.0 | 28.8 | 27.9 | | | | | | | | | | | | | | |
| 28.8 | 30.6 | 29.7 | | | | | | | | | | | | | | |

第　13　表

動物記号　K. N. I

腹毛　Abdominal Hair

| 毛柄幅 (μ) | | | 暗葉幅 (μ) | | | | 明葉幅 (μ) | | | | 暗葉，明葉，幅平均 | | | | 明暗比率 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.8 μ 間隔階級 μ | | 代表値 μ | 最小 μ | 最大 μ | 平均値 μ | 平均数 | 最小 μ | 最大 μ | 平均値 μ | 平均数 | 最小 μ | 最大 μ | 平均値 μ | 平均数 | 暗葉 % | 明葉 % |
| 5.4 | 7.2 | 6.3 | | | | | | | | | | | | | | |
| 7.2 | 9.0 | 8.1 | | | | | | | | | | | | | | |
| 9.0 | 10.8 | 9.9 | | | | | | | | | | | | | | |
| 10.8 | 12.6 | 11.7 | | | | | | | | | | | | | | |
| 12.6 | 14.4 | 13.5 | 17.1 | 22.5 | 18.90 | 4 | | | | | 17.1 | 22.5 | 18.90 | 4 | 100 | 0 |
| 14.4 | 16.2 | 15.3 | 17.1 | 18.9 | 17.10 | 6 | | | | | 17.1 | 18.9 | 17.10 | 6 | 100 | 0 |
| 16.2 | 18.0 | 17.1 | 18.9 | 20.7 | 20.70 | 3 | 22.5 | 26.1 | 24.30 | 4 | 18.9 | 26.1 | 22.50 | 7 | 43− | 57+ |
| 18.0 | 19.8 | 18.9 | 22.5 | 26.1 | 24.30 | 4 | 22.5 | 26.1 | 24.30 | 4 | 22.5 | 26.1 | 24.30 | 8 | 50 | 50 |
| 19.8 | 21.6 | 20.7 | | | | | 24.3 | 40.5 | 35.10 | 3 | 24.3 | 40.5 | 35.10 | 3 | 0 | 100 |
| 21.6 | 23.4 | 22.5 | | | | | 26.1 | 47.7 | 36.90 | 7 | 26.1 | 47.7 | 36.90 | 7 | 0 | 100 |
| 23.4 | 25.2 | 24.3 | | | | | 26.1 | 35.1 | 31.50 | 2 | 26.1 | 35.1 | 31.50 | 2 | 0 | 100 |
| 25.2 | 27.0 | 26.1 | | | | | | | | | | | | | | |
| 27.0 | 28.8 | 27.9 | | | | | | | | | | | | | | |
| 28.8 | 30.6 | 29.7 | | | | | | | | | | | | | | |

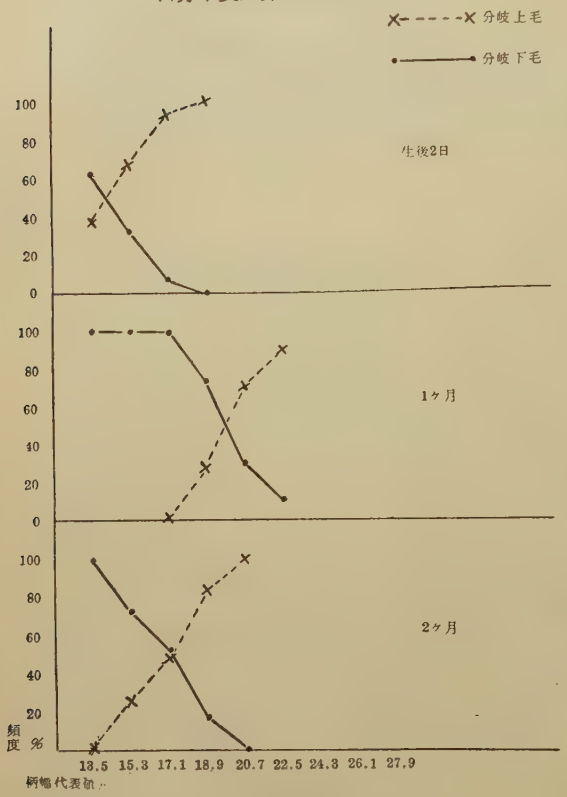
第 14 表　生後，発育過程中の明，暗葉毛分岐域の百分率度数表

| 動物記号→ | K. N. 1. 背毛 | | K. N. 2. 背毛 | | K. N. 3. 背毛 | | K. N. 4. 背毛 | |
|---|---|---|---|---|---|---|---|---|
| 柄幅代表値 μ | 暗葉 % | 明葉 % | 暗葉 % | 明葉 % | 暗葉 % | 明葉 % | 暗葉 % | 明葉 % |
| 11.7 | | | | | | | 100 | 0 |
| 13.5 | 100 | 0 | 100 | 0 | | | 100 | 0 |
| 15.3 | 100 | 0 | 73+ | 27− | 100 | 0 | 100 | 0 |
| 17.1 | 100 | 0 | 52+ | 48− | 100 | 0 | 64+ | 36− |
| 18.9 | 100 | 0 | 17+ | 83− | 96+ | 4− | 29− | 71+ |
| 20.7 | 100 | 0 | 0 | 100 | 83+ | 17− | 8− | 92+ |
| 22.5 | 73− | 27+ | 0 | 100 | 60 | 40 | 0 | 100 |
| 24.3 | 30 | 70 | 0 | 100 | 12− | 88+ | | |
| 26.1 | 11+ | 89− | | | 0 | 100 | | |
| 27.9 | | | | | | | | |

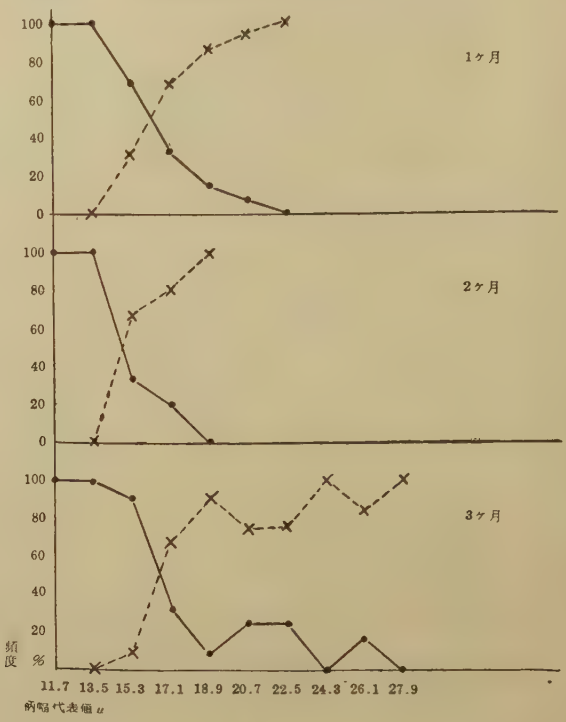
第　15　表

| 動物記号→ | K. N. V−1. 背毛 | | K. N. V−2. 背毛 | | K. N. V−3. 背毛 | | K. N. V−4 背毛 | | K. N. V−6. 背毛 | | K. N. V−8. 背毛 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 柄幅代表値 μ | 暗葉% | 明葉% | 暗葉% | 明葉% | 暗葉% | 明葉% | 暗葉% | 明葉% | 暗葉% | 明葉% | 暗葉% | 明葉% |
| 13.5 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 |
| 15.3 | 69+ | 31− | 33+ | 67− | 91− | 9+ | 100 | 0 | 100 | 0 | 100 | 0 |
| 17.1 | 32• | 68• | 20 | 80 | 33+ | 67− | 95+ | 5− | 97− | 3+ | 96− | 4+ |
| 18.9 | 15− | 85+ | 0 | 100 | 9+ | 91− | 54+ | 46− | 83− | 17+ | 95− | 5+ |
| 20.7 | 7− | 93+ | 0 | 100 | 25• | 75° | 56+ | 44− | 27+ | 73− | 83− | 17+ |
| 22.5 | 0 | 100 | 0 | 100 | 25• | 75° | 50° | 50° | 45° | 55° | 73+ | 27− |
| 24.3 | 0 | 100 | 0 | 100 | 0 | 100 | 50° | 50° | 33+ | 67− | 33+ | 67− |
| 26.1 | 0 | 100 | 0 | 120 | 17− | 83+ | 29− | 71+ | 9° | 91° | 43− | 57+ |
| 27.9 | | | | | | | | | 0 | 100 | 17− | 83+ |

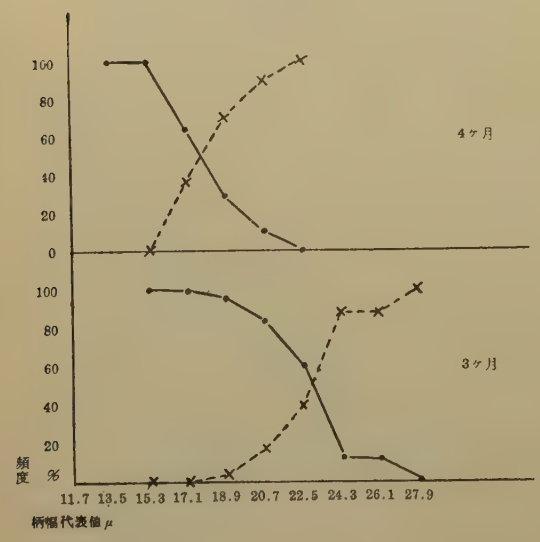
第9図 一腹出生仔獣の分岐域（各月別個体）
百分率度数分岐折線の図



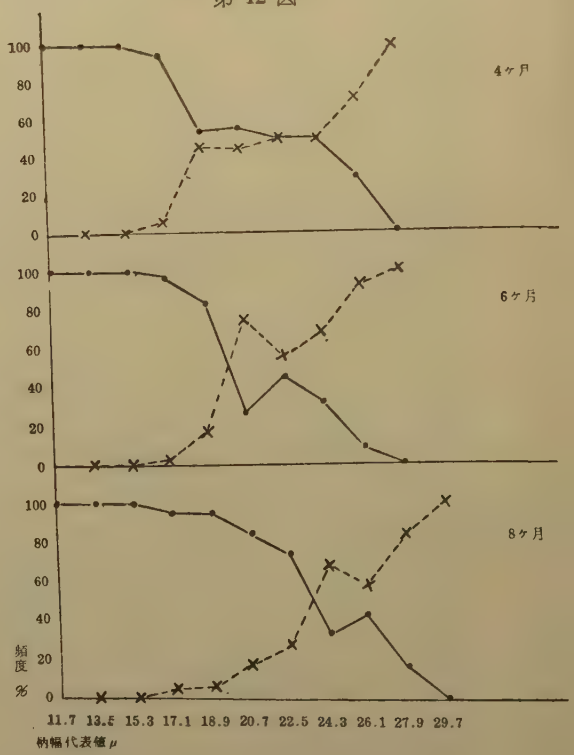

第11図 百分率度数分岐折線の図（KN V.1～8）




第 10 図




第 12 図



第16表　毛叢（上毛＋下毛）の度数分布表とその分岐表（第13図に表わす）

Animal no. K. N. 4 背毛

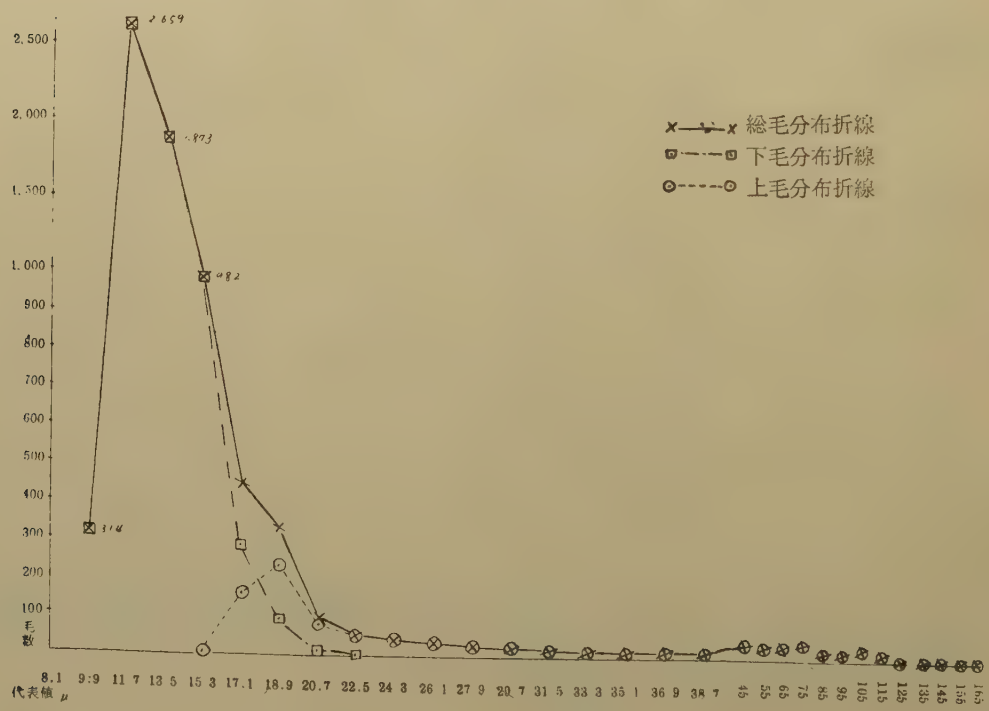
| 1.8 μ 級間 | | | | 10 μ 級間 | | | | 観察総数に対する | | 小型毛葉の明暗比 | | 下毛，分岐毛，上毛の分離 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 階級 μ | | 代表値 μ | 頻度 | 階級 μ | | 代表値 μ | 頻度 | 級間 1.8μ % | 級間 10μ % | 暗葉 % | 明葉 % | 下毛と分岐毛 | | 分岐上毛と上毛 | | 下毛と分岐毛の頻度 % |
| 5.4 | 7.2 | 6.3 | | | | | | | | | | | | | | |
| 7.2 | 9.0 | 8.1 | | 0 | 10 | 5 | | | | | | 下 | | | | |
| 9.0 | 10.8 | 9.9 | 314 | | | | | | | | | | 314 | | | |
| 10.8 | 12.6 | 11.7 | 2,659 | | | | | | | | | | 2,659 | | | |
| 12.6 | 14.4 | 13.5 | 1,873 | | | | | | | | | 毛 | 1,873 | | | |
| 14.4 | 16.2 | 15.3 | 982 | | | | | | | | | | 982 | | | |
| 16.2 | 18.0 | 17.1 | 438 | | | | | | | | | | 280 | 158 | | 分岐上毛 |
| 18.0 | 19.8 | 18.9 | 322 | 10 | 20 | 15 | 6,588 | | | | | 分岐下毛 | 94 | 228 | | |
| 19.8 | 21.6 | 20.7 | 82 | | | | | | | | | | 8 | 74 | | |
| 21.6 | 23.4 | 22.5 | 52 | | | | | | | | | | | 52 | | 上毛↓ |
| 23.4 | 25.2 | 24 3 | 43 | | | | | | | | | | | 43 | | |
| 25.2 | 27.0 | 26.1 | 35 | | | | | | | | | | | 35 | | |
| 27.0 | 28.8 | 27.9 | 24 | 20 | 30 | 25 | 236 | | | | | | | 24 | | |
| 28.8 | 30.6 | 29.7 | 16 | | | | | | | | | | | 16 | | |
| 30.6 | 32.4 | 31.5 | 14 | | | | | | | | | | | 14 | | |
| 32.4 | 34.2 | 33.3 | 8 | | | | | | | | | | | 8 | | |
| 34.2 | 36.0 | 35.1 | 9 | | | | | | | | | | | 9 | | |
| 36.0 | 37.8 | 36.9 | 11 | | | | | | | | | | | 11 | | |
| 37.8 | 39.6 | 38.7 | 9 | 30 | 40 | 35 | 67 | | | | | | | 9 | | |
| | | | | 40 | 50 | 45 | 40 | | | | | | | 40 | | |
| | | | | 50 | 60 | 55 | 28 | | | | | | | 28 | | |
| | | | | 60 | 70 | 65 | 31 | | | | | | | 31 | | |
| | | | | 70 | 80 | 75 | 42 | | | | | | | 42 | | |
| | | | | 80 | 90 | 85 | 20 | | | | | | | 20 | | |
| | | | | 90 | 100 | 95 | 14 | | | | | | | 14 | | |
| | | | | 100 | 110 | 105 | 23 | | | | | | | 23 | | |
| | | | | 110 | 120 | 115 | 14 | | | | | | | 14 | | |
| | | | | 120 | 130 | 125 | 3 | | | | | | | 3 | | |
| | | | | 130 | 140 | 135 | 3 | | | | | | | 3 | | |
| | | | | 140 | 150 | 145 | 2 | | | | | | | 2 | | |
| | | | | 150 | 160 | 155 | 0 | | | | | | | 0 | | |
| | | | | 160 | 170 | 165 | 2 | | | | | | | 2 | | |
| | | | | 170 | 180 | 175 | 0 | | | | | | | 0 | | |
| | | | | | | | 7,113 | | | | | | 6,210 | 903 | | |
| | | | | | | | | | | | | | 87+ % | 13− % | | |

第17表 毛柄と毛葉との相関表

(1) 小型暗葉毛（下毛＋分岐下毛）

| 動物記号→ | K. N. 1. 背毛 | | K. N. 2. 背毛 | |
|---|---|---|---|---|
| 柄幅級間 1.8μ. 代表値μ | 葉幅観察値 (y) μ | 期待値 (Y) μ | 葉幅観察値 (y) μ | 期待値 (Y) μ |
| 11.7 | 13.86 | 13.70 | | |
| 13.5 | 17.33 | 16.59 | 15.30 | 16.14 |
| 15.3 | 19.30 | 19.48 | 19.18 | 18.18 |
| 17.1 | 21.65 | 22.37 | 20.70 | 20.22 |
| 18.9 | 23.85 | 25.26 | 21.60 | 22.26 |
| 20.7 | 29.10 | 28.15 | | |
| 22.5 | 31.50 | 31.04 | | |
| | K. N. 3. 背毛 | | K. N. 4. 背毛 | |
| 11.7 | | | 12.45 | 13.14 |
| 13.5 | | | 14.85 | 15.65 |
| 15.3 | 16.50 | 18.11 | 19.65 | 18.16 |
| 17.1 | 21.13 | 20.19 | 21.50 | 20.67 |
| 18.9 | 23.66 | 22.27 | 23.73 | 23.18 |
| 20.7 | 24.54 | 24.85 | 24.30 | 25.74 |
| 22.5 | 26.10 | 26.43 | | |
| 24.3 | 27.90 | 28.51 | | |

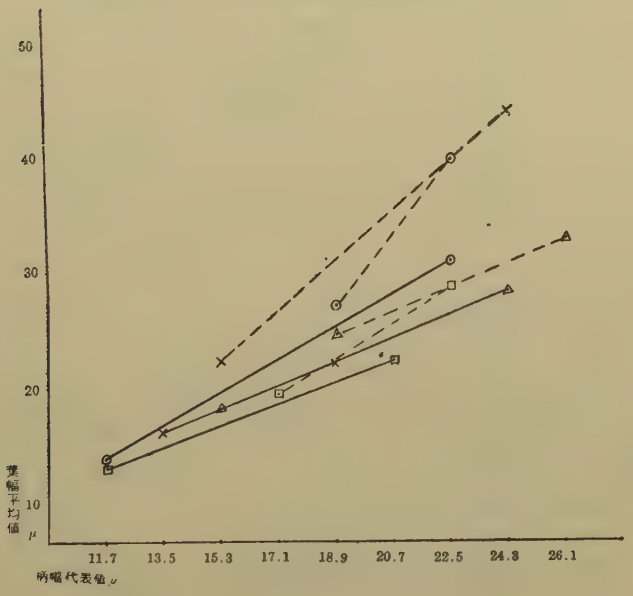
第 13 図 上毛と下毛の度数分布図とその分岐図



第18表　毛柄と毛葉との相関表
（2）　小型明葉毛（分岐上毛＋小型第2上毛）

| 動物記号→ | K. N. 1. 背毛 | | K. N. 2. 背毛 | |
|---|---|---|---|---|
| 柄幅級間 1.8 μ. 代表値 μ | 葉幅観察値 (y) μ | 期待値 (Y) μ | 葉幅観察値 (y) μ | 期待値 (Y) μ |
| 15.3 | | | 23.79 | 22.69 |
| 17.1 | | | 25.45 | 27.01 |
| 18.9 | 26.10 | 27.32 | 31.31 | 31.33 |
| 20.7 | 36.18 | 33.74 | 37.62 | 35.65 |
| 22.5 | 38.93 | 40.16 | 36.90 | 39.97 |
| 24.3 | | | 45.90 | 44.29 |
| | K. N. 3. 背毛 | | K. N. 4. 背毛 | |
| 17.1 | | | 23.19 | 22.73 |
| 18.9 | 24.30 | 24.63 | 24.38 | 24.74 |
| 20.7 | 27.90 | 26.76 | 26.05 | 26.75 |
| 22.5 | 27.60 | 28.89 | 29.34 | 28.76 |
| 24.3 | 31.50 | 31.02 | | |
| 26.1 | 33.14 | 33.15 | | |

第 14 図　分岐域の暗葉幅と明葉幅の関係図 (K. N. I—4)



第19表 毛柄と毛葉との相関表
(3) 小型暗葉毛(下毛+分岐下毛)

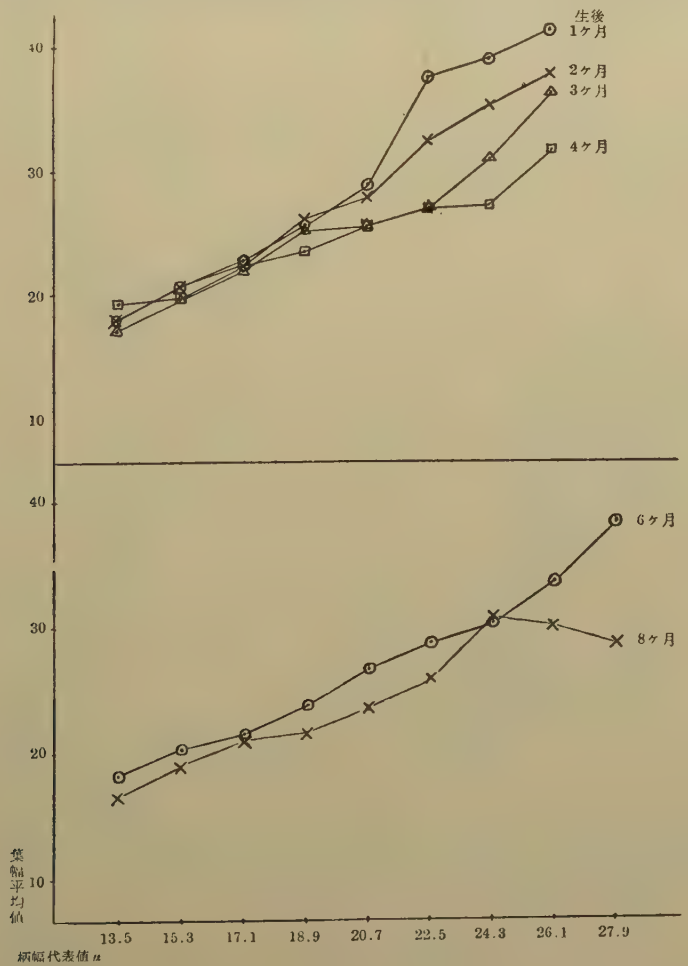
| 動物記号→ | K. N. V—1. 背毛 | | K. N. V—2. 背毛 | |
|---|---|---|---|---|
| 柄幅級間 1.8μ. 代表値μ | 葉幅観察値(y)μ | 期待値(Y)μ | 葉幅観察値(y)μ | 期待値(Y)μ |
| 13.5 | 17.82 | 18.19 | 17.91 | 18.10 |
| 15.3 | 19.32 | 19.27 | 18.70 | 18.30 |
| 17.1 | 21.37 | 20.34 | 18.30 | 18.49 |
| 18.9 | 20.70 | 21.41 | | |
| 20.7 | 22.50 | 22.49 | | |
| | K. N. V—3. 背毛 | | K. N. V—4. 背毛 | |
| 13.5 | 17.22 | 18.00 | 19.26 | 19.00 |
| 15.3 | 19.62 | 19.47 | 19.74 | 20.41 |
| 17.1 | 22.14 | 20.94 | 22.03 | 21.81 |
| 18.9 | 22.50 | 22.41 | 22.63 | 23.22 |
| 20.7 | 23.40 | 23.88 | 25.70 | 24.63 |
| 22.5 | 25.20 | 25.35 | 26.55 | 25.04 |
| 24.3 | | | 26.70 | 27.44 |
| 26.1 | | | 28.80 | 28.85 |
| | K. N. V—6. 背毛 | | K. N. V—8. 背毛 | |
| 13.5 | 18.17 | 18.49 | 16.50 | 17.25 |
| 15.3 | 20.28 | 20.01 | 18.90 | 18.63 |
| 17.1 | 21.37 | 21.53 | 20.54 | 20.01 |
| 18.9 | 23.03 | 23.05 | 21.50 | 21.39 |
| 20.7 | 24.30 | 24.57 | 22.88 | 22.77 |
| 22.5 | 26.70 | 26.09 | 23.32 | 24.15 |
| 24.3 | 28.80 | 27.61 | 25.50 | 25.53 |
| 26.1 | 27.90 | 29.31 | 29.70 | 26.91 |
| 27.9 | | | 26.10 | 28.29 |

第20表 毛柄と毛葉との相関表
(4) 小型明葉毛(分岐上毛+小型第2上毛)

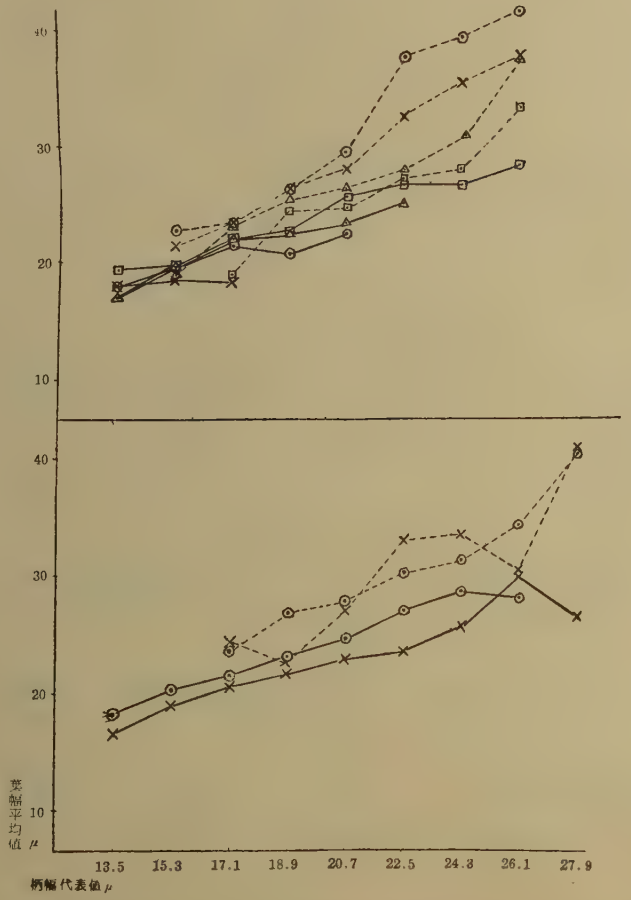
| 動物記号→ | K. N. V—1 背毛 | | K. N. V—2 背毛 | |
|---|---|---|---|---|
| 柄幅級間 1.8μ 代表値μ | 葉幅観察値(y)μ | 期待値(Y)μ | 葉幅観察値(y)μ | 期待値(Y)μ |
| 15.3 | 22.72 | 20.81 | 21.44 | 20.78 |
| 17.1 | 23.55 | 24.42 | 23.50 | 23.63 |
| 18.9 | 26.40 | 28.03 | 26.26 | 26.48 |
| 20.7 | 29.57 | 31.64 | 28.06 | 29.33 |
| 22.5 | 37.80 | 35.25 | 32.58 | 32.18 |
| 24.3 | 39.60 | 38.86 | 35.70 | 35.03 |
| 26.1 | 41.85 | 42.47 | 37.80 | 37.88 |

| | K. N. V—3 背毛 | | K. N. V—4 背毛 | |
|---|---|---|---|---|
| 15.3 | 18.90 | 19.34 | | |
| 17.1 | 23.40 | 21.99 | 18.90 | 20.15 |
| 18.9 | 25.56 | 24.64 | 24.46 | 22.59 |
| 20.7 | 26.40 | 27.29 | 25.32 | 25.03 |
| 22.5 | 27.90 | 29.94 | 27.45 | 27.47 |
| 24.3 | 31.05 | 32.59 | 27.90 | 29.91 |
| 26.1 | 37.80 | 35.24 | 33.52 | 32.35 |
| | K. N. V—6 背毛 | | K. N. V—8 背毛 | |
| 17.1 | 23.40 | 23.16 | 24.30 | 22.39 |
| 18.9 | 26.82 | 25.61 | 22.50 | 24.98 |
| 20.7 | 27.68 | 28.06 | 26.82 | 27.57 |
| 22.5 | 30.19 | 30.51 | 32.80 | 30.16 |
| 24.3 | 31.05 | 32.96 | 33.30 | 32.75 |
| 26.1 | 34.20 | 35.41 | 30.15 | 35.34 |
| 27.9 | 40.24 | 37.86 | 41.22 | 37.93 |

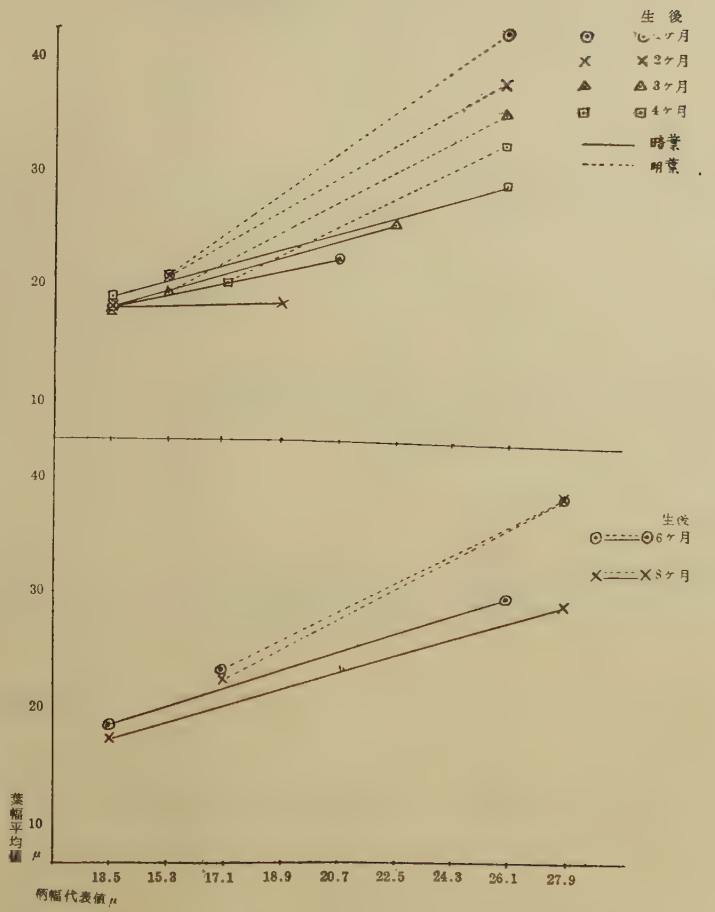
第 15 図　分岐域の柄幅と葉幅の関係図（K. N. V—1→8）明葉と暗葉混合



第16図　分岐域の柄幅と葉幅の関係図（K. N.　V−1→8）明葉と暗葉の分離




第17図　分岐域の柄幅と葉幅の関係図（K. N.　V−1→8）
明葉と暗葉の分離



第21表　下毛＋分岐下毛

Animal no. K. N. V—8　背　毛

| 毛柄幅（正幅） Breadth of hair stalk (a) | | | 観察頻度 |
|---|---|---|---|
| 1.8 μ 級間 1.8 μ Class interval | | 代表値 Mean of Class μ | Observed frequency |
| 5.4 | 7.2 | 6.3 | |
| 7.2 | 9.0 | 8.1 | 21 |
| 9.0 | 10.8 | 9.9 | 142 |
| 10.8 | 12.6 | 11.7 | 155 |
| 12.6 | 14.4 | 13.5 | 73 |
| 14.4 | 16.2 | 15.3 | 48 |
| 16.2 | 18.0 | 17.1 | 21 |
| 18.0 | 19.8 | 18.9 | 11 |
| 19.8 | 21.6 | 20.7 | 10 |
| 21.6 | 23.4 | 22.5 | 8 |
| 23.4 | 25.2 | 24.3 | 1 |
| 25.2 | 27.0 | 26.1 | 1 |
| 27.0 | 28.8 | 27.9 | 1 |
| 28.8 | 30.6 | 29.7 | |
| 30.6 | 32.4 | 31.5 | |
| 32.4 | 34.2 | 33.3 | |

| 統計量 Statistics | | |
|---|---|---|
| 相加平均 $\bar{x}$ | Arithmetic Mean | 12.48 μ |
| 偏差平方和 $S(x-\bar{x})^2$ | Sum of squares of deviation | 4843.5366 |
| 自由度 N | Degree of freedom | 491 |
| 分散 $S^2$ | Variance | 9.8646 |
| 標準偏差 S（標本） | Standard deviation | 3.14 μ |
| 変異係数 $s/x\times100$ | Coefficient of variation per cent | 25.16 % |

Animal no. K. N. V—8　腹　毛

| 毛柄幅（正幅） Breadth of hair stalk (a) | | | 観察頻度 |
|---|---|---|---|
| 1.8 μ 級間 1.8 μ Class interval | | 代表値 Mean of Class μ | Observed frequency |
| 5.4 | 7.2 | 6.3 | 1 |
| 7.2 | 9.0 | 8.1 | 82 |
| 9.0 | 10.8 | 9.9 | 297 |
| 10.8 | 12.6 | 11.7 | 110 |
| 12.6 | 14.4 | 13.5 | 16 |
| 14.4 | 16.2 | 15.3 | 2 |
| 16.2 | 18.0 | 17.1 | 0 |
| 18.0 | 19.8 | 18.9 | 4 |
| 19.8 | 21.6 | 20.7 | 1 |
| 21.6 | 23.4 | 22.5 | 0 |
| 23.4 | 25.2 | 24.3 | 0 |
| 25.2 | 27.0 | 26.1 | 1 |
| 27.0 | 28.8 | 27.9 | |
| 28.8 | 30.6 | 29.7 | |
| 30.6 | 32.4 | 31.5 | |
| 32.4 | 34.2 | 33.3 | |

| 統計量 | | |
|---|---|---|
| 相加平均 $\bar{x}$ | Arithmetic Mean | 10.25 μ |
| 偏差平方和 $S(x-\bar{x})^2$ | Sum of squares of deviation | 1542.0194 |
| 自由度 N | Degree of freedom | 513 |
| 分散 $S^2$ | Variance | 3.0058 |
| 標準偏差 S（標本） | Stondard deviation | 1.73 μ |
| 変異係数 $s/x\times100$ | Coefficient of variation per cent | 16.87 % |

第22表 幼獣の発育過程中における小型暗葉毛の柄副統計量

背 毛

| No. | 性 別 | 動物記号 | A / B | 分岐下毛＋下毛 / 下 毛 | 相加平均 ($\bar{x}$) μ | 分 散 ($S^2$) | 標準偏差 (S) μ | 変異係数 ($s/\bar{x}\times100$)% |
|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | K. N. 0 | A | | 19.22 | 13.6189 | 3.69 | 19.20 |
| | | | B | | — | — | — | — |
| 2 | ♂ | K. N. 1 | A | | 14.43 | 2.0599 | 1.44 | 10.00 |
| | | | B | | 14.25 | 1.1576 | 1.08 | 7.57 |
| 3 | ♂ | K. N. 2 | A | | 14.23 | 1.6728 | 1.29 | 9.06 |
| | | | B | | 13.33 | 0.2833 | 0.53 | 3.98 |
| 4 | ♂ | K. N. 3 | A | | 13.87 | 5.7732 | 2.40 | 17.30 |
| | | | B | | 13.60 | 2.7686 | 1.66 | 12.20 |
| 5 | ♂ | K. N. 4 | A | | 13.13 | 3.6367 | 1.91 | 14.54 |
| | | | B | | 12.79 | 2.2212 | 1.49 | 11.65 |
| 6 | ♀ | K. N. V—1 | A | | 11.67 | 2.9552 | 1.72 | 14.73 |
| | | | B | | 11.40 | 1.7852 | 1.34 | 11.75 |
| 7 | ♀ | K. N. V—2 | A | | 11.73 | 2.4216 | 1.56 | 13.29 |
| | | | B | | 11.61 | 1.9225 | 1.39 | 11.97 |
| 8 | ♀ | K. N. V—3 | A | | 11.19 | 3.5962 | 1.90 | 16.97 |
| | | | B | | 10.75 | 2.2181 | 1.49 | 13.86 |
| 9 | ♀ | K. N. V—4 | A | | 11.84 | 8.0881 | 2.84 | 23.99 |
| | | | B | | 11.16 | 3.1900 | 1.79 | 16.03 |
| 10 | ♀ | K. N. V—6 | A | | 11.55 | 6.9031 | 2.63 | 22.77 |
| | | | B | | 11.11 | 3.8506 | 1.96 | 17.64 |
| 11 | ♀ | K. N. V—8 | A | | 12.48 | 9.8646 | 3.14 | 25.16 |
| | | | B | | 11.64 | 3.6283 | 1.90 | 16.32 |

第23表 背毛（下毛＋分岐下毛）の度数分布を鑑別規準（K）により決定する計算表

動物記号 K. N. V—8 背 毛

| 柄幅 1.8 μ 間隔 代表値 (μ) | 偏 (x′) | 度 数 (x) | 第1次乗積能率 (fx′) | 第2次乗積能率 (fx′²) | 第3次乗積能率 (fx′³) | 第4次乗積能率 (fx′⁴) |
|---|---|---|---|---|---|---|
| 8.1 | − 2 | 21 | − 42 | 84 | − 168 | 336 |
| 9.9 | − 1 | 142 | − 142 | 142 | − 142 | 142 |
| 11.7 | 0 | 155 | 0 | 0 | 0 | 0 |
| 13.5 | 1 | 73 | 73 | 73 | 73 | 73 |
| 15.3 | 2 | 48 | 96 | 192 | 384 | 768 |
| 17.1 | 3 | 21 | 63 | 189 | 567 | 1701 |
| 18.9 | 4 | 11 | 44 | 176 | 704 | 2816 |
| 20.7 | 5 | 10 | 50 | 250 | 1250 | 6250 |
| 22.5 | 6 | 8 | 48 | 288 | 1728 | 10368 |
| 24.3 | 7 | 1 | 7 | 49 | 343 | 2401 |
| 26.1 | 8 | 1 | 8 | 64 | 512 | 4096 |
| 27.9 | 9 | 1 | 9 | 81 | 729 | 6561 |
| 計 | — | 492 | 214 | 1588 | 5980 | 35512 |

$x'=m'_1=\frac{214}{492}=0.4349$

$m'_2=\frac{1588}{492}=3.2276$

$m'_3=\frac{5980}{492}=12.1544$

$m'_4=\frac{35512}{492}=72.1788$

$v_2=m'_2-\bar{x}'^2=3.0385$

$v_3=m'_3-3v_2\bar{x}'-\bar{x}'^3=8.1080$

$v_4=m'_4-4v_3\bar{x}'-6v_2\bar{x}'^2-\bar{x}'^4=54.5379$

$\beta_1=\frac{v_3^2}{v_2^3}=2.3434,\quad \beta_2=\frac{v_4}{v_2^2}=5.9071$

$K=\beta_1(\beta_2+3)^2/4(4\beta_2-3\beta_1)(2\beta_2-3\beta_1-6)=-0.2585$

K は負であるから与えられた度数分布に適合する曲線は，ピアソン系の第1型である．

仮想平均　11.7 $\mu$，級間1.8 $\mu$

$\bar{x}=11.7+1.8\bar{x}'=11.7+1.8\times0.4349$

$=12.4828\fallingdotseq12.48\,\mu$

第24表　幼獣の充育過程中における小型暗葉毛の柄副統計量

腹　毛

| No. | 性　別 | 動物記号 | A / B | 分岐下毛＋下毛 / 下　毛 | 相加平均 ($\bar{x}$) $\mu$ | 分　散 ($S^2$) | 標準偏差 ($S$) $\mu$ | 変異係数 ($s/\bar{x}\times100$)% |
|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | K. N. 0 | A | | 19.22 | 13.6189 | 3.69 | 19.20 |
| | | | B | | — | — | — | — |
| 2 | ♂ | K. N. 1 | A | | 14.43 | 2.0599 | 1.44 | 10.00 |
| | | | B | | 14.25 | 1.1576 | 1.08 | 7.57 |
| 3 | ♂ | K. N. 2 | A | | 14.23 | 1.6728 | 1.29 | 9.06 |
| | | | B | | 13.33 | 0.2833 | 0.53 | 3.98 |
| 4 | ♂ | K. N. 3 | A | | 13.87 | 5.7732 | 2.40 | 17.30 |
| | | | B | | 13.60 | 2.7686 | 1.66 | 12.20 |
| 5 | ♂ | K. N. 4 | A | | 13.13 | 3.6367 | 1.91 | 14.54 |
| | | | B | | 12.79 | 2.2212 | 1.49 | 11.65 |
| 6 | ♀ | K. N. V—1 | A | | 11.67 | 2.9552 | 1.72 | 14.73 |
| | | | B | | 11.40 | 1.7852 | 1.34 | 11.75 |
| 7 | ♀ | K. N. V—2 | A | | 11.73 | 2.4216 | 1.56 | 13.29 |
| | | | B | | 11.61 | 1.9225 | 1.39 | 11.97 |
| 8 | ♀ | K. N. V—3 | A | | 11.19 | 3.5962 | 1.90 | 16.97 |
| | | | B | | 10.75 | 2.2181 | 1.49 | 13.86 |
| 9 | ♀ | K. N. V—4 | A | | 11.84 | 8.0881 | 2.84 | 23.99 |
| | | | B | | 11.16 | 3.1900 | 1.79 | 16.03 |
| 10 | ♀ | K. N. V—6 | A | | 11.55 | 6.9031 | 2.63 | 22.77 |
| | | | B | | 11.11 | 3.8506 | 1.96 | 17.64 |
| 11 | ♀ | K. N. V—8 | A | | 12.48 | 9.8646 | 3.14 | 25.16 |
| | | | B | | 11.64 | 3.6283 | 1.90 | 16.32 |

Animal no. K.N. V—8

## 第25表 上毛の幹幅比較表

### 背部上毛

| 毛柄幅 10μ間隔 階級(μ) | | 代表値(μ) | 明葉毛 毛頸幅(μ) 最小 | 最大 | 平均値 | 明葉毛 毛葉幅(μ) 最小 | 最大 | 平均値 | 平均数 | 暗葉毛 毛頸幅 最小 | 最大 | 平均値 | 暗葉毛 毛葉幅(μ) 最小 | 最大 | 平均値 | 平均数 | 明葉,暗葉平均 毛頸幅(μ) 最小 | 最大 | 平均値 | 明葉,暗葉平均 毛葉幅(μ) 最小 | 最大 | 平均値 | 平均数 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 20 | 15 | | | | 22.5 | 24.3 | 23.4 | 2 | | | | | | | | | | | 22.5 | 24.3 | 23.4 | 2 |
| 20 | 30 | 25 | | | | 22.5 | 58.5 | 32.9 | 24 | | | | | | | | | | | 22.5 | 58.5 | 32.9 | 24 |
| 30 | 40 | 35 | | | | 31.5 | 65.7 | 45.9 | 22 | | | | | | | | | | | 31.5 | 65.7 | 45.9 | 22 |
| 40 | 50 | 45 | | | | 56.7 | 80.1 | 67.5 | 7 | | | | | | | | | | | 56.7 | 80.1 | 67.5 | 7 |
| 50 | 60 | 55 | 27.9 | 47.7 | 38.7 | 60.3 | 128.7 | 81.9 | 19 | | | | | | | | 27.9 | 47.7 | 38.7 | 60.3 | 128.7 | 81.9 | 19 |
| 60 | 70 | 65 | 40.5 | 44.1 | 44.1 | 76.5 | 128.7 | 105.3 | 9 | | | | | | | | 40.5 | 44.1 | 44.1 | 76.5 | 128.7 | 105.3 | 9 |
| 70 | 80 | 75 | 38.7 | 62.1 | 51.3 | 87.3 | 155.7 | 117.9 | 14 | | | | | | | | 38.7 | 62.1 | 51.3 | 87.3 | 155.7 | 117.9 | 14 |
| 80 | 90 | 85 | 45.9 | 83.7 | 65.7 | 99.9 | 173.7 | 141.3 | 32 | — | 80.1 | 80.1 | — | 152.1 | 152.1 | 1 | 45.9 | 83.7 | 65.7 | 99.9 | 179.1 | 143.1 | 33 |
| 90 | 100 | 95 | 38.7 | 98.1 | 74.7 | 98.1 | 179.1 | 144.9 | 43 | 62.1 | 85.5 | 76.5 | 128.7 | 179.1 | 155.7 | 16 | 38.7 | 98.1 | 74.7 | 99.0 | 179.1 | 146.7 | 59 |
| 100 | 110 | 105 | 56.7 | 89.1 | 71.1 | 125.1 | 179.1 | 152.1 | 41 | 63.9 | 87.3 | 76.5 | 143.1 | 173.7 | 155.7 | 27 | 56.7 | 89.1 | 72.9 | 125.1 | 179.1 | 152.1 | 68 |
| 110 | 120 | 115 | 63.9 | 85.5 | 74.7 | 139.5 | 184.5 | 157.5 | 12 | — | — | 78.3 | 135.9 | 164.7 | 152.1 | 9 | — | — | 76.5 | 135.9 | 184.5 | 155.7 | 21 |
| 120 | 130 | 125 | | | | | | | | | | | | | | | | | | | | | |
| 130 | 140 | 135 | | | | | | | | | | | | | | | | | | | | | |
| 140 | 150 | 145 | | | | | | | | | | | | | | | | | | | | | |

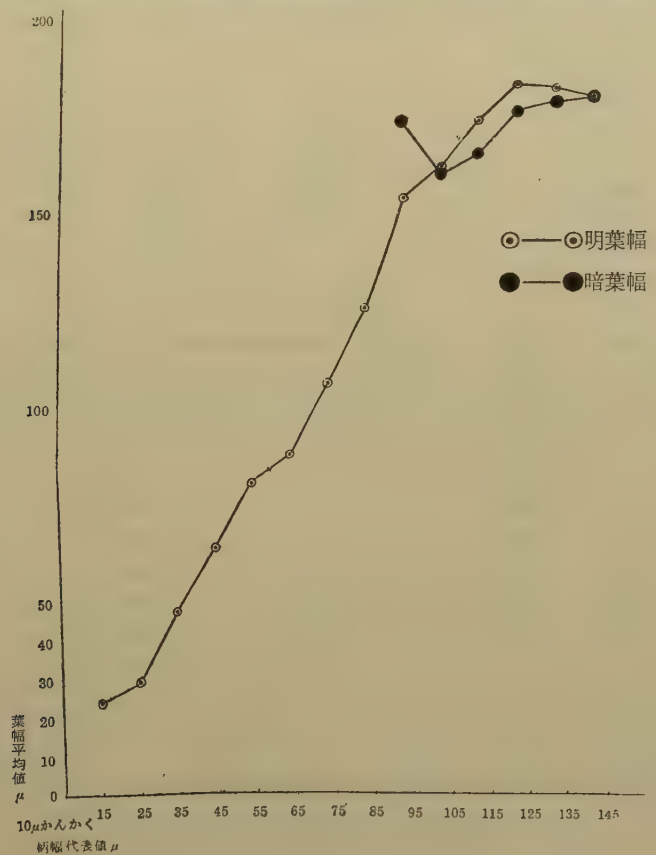
### 腹部上毛

| 毛柄幅 階級μ | | 代表値 | 毛頸幅(μ) 最小 | 最大 | 平均値 | 毛葉幅(μ) 最小 | 最大 | 平均値 | 平均数 |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 20 | 15 | — | 15.3 | 15.3 | 20.7 | 38.7 | 29.7 | 7 |
| 20 | 30 | 25 | 17.1 | 22.5 | 18.9 | 26.1 | 49.5 | 35.1 | 5 |
| 30 | 40 | 35 | 26.1 | 35.1 | 29.7 | 60.3 | 92.7 | 83.7 | 7 |
| 40 | 50 | 45 | 26.1 | 44.1 | 33.3 | 65.7 | 99.9 | 81.9 | 12 |
| 50 | 60 | 55 | 35.1 | 42.3 | 36.9 | 62.1 | 103.5 | 74.7 | 5 |
| 60 | 70 | 65 | 35.1 | 47.7 | 45.9 | 85.5 | 119.7 | 108.9 | 11 |
| 70 | 80 | 75 | 38.7 | 72.9 | 51.3 | 92.7 | 135.9 | 112.5 | 15 |
| 80 | 90 | 85 | 60.3 | 76.5 | 71.1 | 107.1 | 125.1 | 116.1 | 3 |
| 90 | 100 | 95 | | | | | | | |

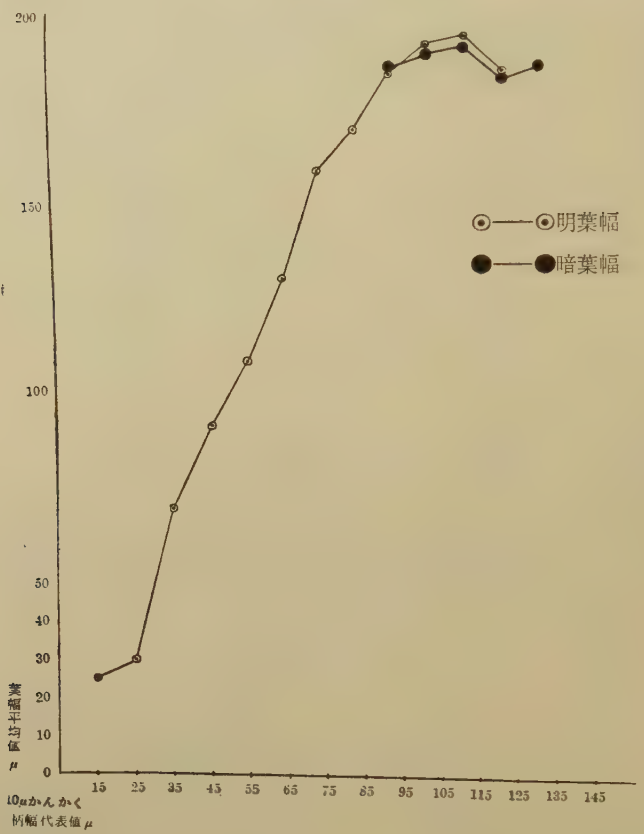
第 26 表

| 動物記号 | | | K. N. A | | K. N. B | |
|---|---|---|---|---|---|---|
| 柄幅代表値 μ | | | 明葉毛 葉幅平均値 μ | 暗葉毛 葉幅平均値 μ | 明葉毛 葉幅平均値 μ | 暗葉毛 葉幅平均値 μ |
| 10 | 20 | 15 | 24.3 | | 22.5 | |
| 20 | 30 | 25 | 29.7 | | 29.7 | |
| 30 | 40 | 35 | 47.7 | | 69.3 | |
| 40 | 50 | 45 | 63.9 | | 90.9 | |
| 50 | 60 | 55 | 80.1 | | 108.9 | |
| 60 | 70 | 65 | 87.3 | | 130.5 | |
| 70 | 80 | 75 | 105.3 | | 159.3 | |
| 80 | 90 | 85 | 125.1 | | 170.1 | |
| 90 | 100 | 95 | 153.9 | 173.7 | 184.5 | 186.3 |
| 100 | 110 | 105 | 161.1 | 159.3 | 191.7 | 189.9 |
| 110 | 120 | 115 | 173.7 | 164.7 | 193.5 | 191.7 |
| 120 | 130 | 125 | 182.7 | 175.5 | 184.5 | 182.7 |
| 130 | 140 | 135 | 180.9 | 177.3 | | 186.3 |
| 140 | 150 | 145 | 179.1 | 179.1 | | |

第 18 図　上毛の柄幅と葉幅の関係図（N. A）明葉と暗葉の分離



第 19 図　上毛の柄幅と葉幅の関係図（N. B）明葉と暗葉の分離




第 27 表

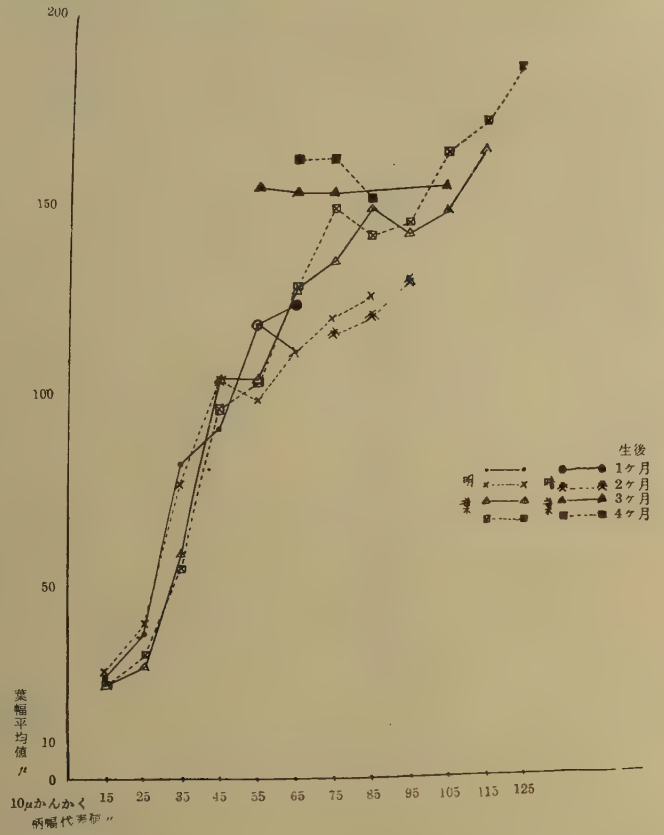
| 動物記号 | | | K. N. 1 背上毛 | | K. N. 2 背上毛 | |
|---|---|---|---|---|---|---|
| 柄幅 代表値 μ | | | 明葉毛 葉幅平均値 μ | 暗葉毛 葉幅平均値 μ | 明葉毛 葉幅平均値 μ | 暗葉毛 葉幅平均値 μ |
| 10 | 20 | 15 | 26.1 | | 26.9 | |
| 20 | 30 | 25 | 37.5 | | 40.2 | |
| 30 | 40 | 35 | 81.9 | | 76.5 | |
| 40 | 50 | 45 | 90.9 | | 103.5 | |
| 50 | 60 | 55 | 117.9 | 117.9 | 98.1 | |
| 60 | 70 | 65 | 110.7 | 123.3 | 110.7 | |
| 70 | 80 | 75 | | | 119.7 | 116.1 |
| 80 | 90 | 85 | | | 125.1 | 119.7 |
| 90 | 100 | 95 | | | | 128.7 |
| 100 | 110 | 105 | | | | |

| | | | K. N. 3 背上毛 | | K. N. 4 背上毛 | |
|---|---|---|---|---|---|---|
| 10 | 20 | 15 | 24.3 | | 23.8 | |
| 20 | 30 | 25 | 28.8 | | 32.3 | |
| 30 | 40 | 35 | 58.5 | | 54.9 | |
| 40 | 50 | 45 | 103.5 | | 96.3 | |
| 50 | 60 | 55 | 103.5 | 153.9 | 92.7 | |
| 60 | 70 | 65 | 126.8 | 152.1 | 128.7 | 161.1 |
| 70 | 80 | 75 | 134.1 | 152.1 | 148.5 | 161.1 |
| 80 | 90 | 85 | 148.5 | | 141.3 | 150.3 |
| 90 | 100 | 95 | 141.3 | | 144.9 | |
| 100 | 110 | 105 | 157.5 | 153.9 | 162.9 | |
| 110 | 120 | 115 | 164.7 | | 170.1 | |
| 120 | 130 | 125 | | | 184.5 | |

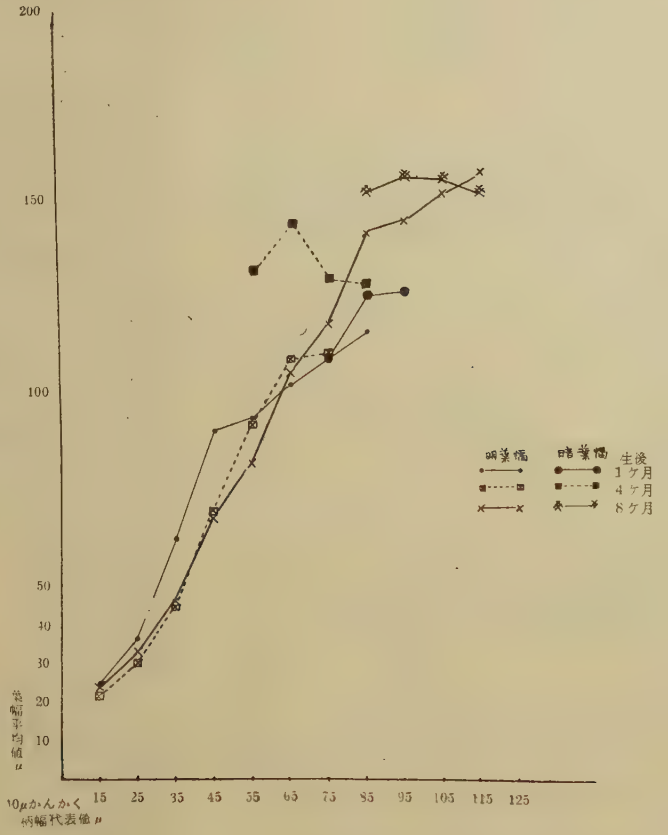
第 28 表

| 動物記号 | K. N. V—1 背上毛 | | K. N. V—4 背上毛 | | K. N. V—8 背上毛 | |
|---|---|---|---|---|---|---|
| 柄幅代表値 μ | 明葉幅平均値 μ | 暗葉幅平均値 μ | 明葉幅平均値 μ | 暗葉幅平均値 μ | 明葉幅平均値 μ | 暗葉幅平均値 μ |
| 15 | 24.2 | | 21.7 | | 23.4 | |
| 25 | 36.8 | | 30.2 | | 32.9 | |
| 35 | 62.1 | | 44.5 | | 45.9 | |
| 45 | 90.2 | | 69.2 | | 67.5 | |
| 55 | 93.6 | | 91.8 | 131.7 | 81.9 | |
| 65 | 102.3 | | 108.7 | 143.8 | 105.3 | |
| 75 | | 108.9 | 109.9 | 129.6 | 117.9 | |
| 85 | 116.1 | 125.6 | | 128.1 | 141.3 | 152.1 |
| 95 | | 126.8 | | | 144.9 | 155.7 |
| 105 | | | | | 152.1 | 155.7 |
| 115 | | | | | 157.5 | 152.1 |
| 125 | | | | | | |

第20図　上毛の柄幅と葉幅の関係図（KN 1→4）明葉と暗葉の分離




第21図　上毛の柄幅と葉幅の関係図（KN V—1→4→8）明葉と暗葉の分離

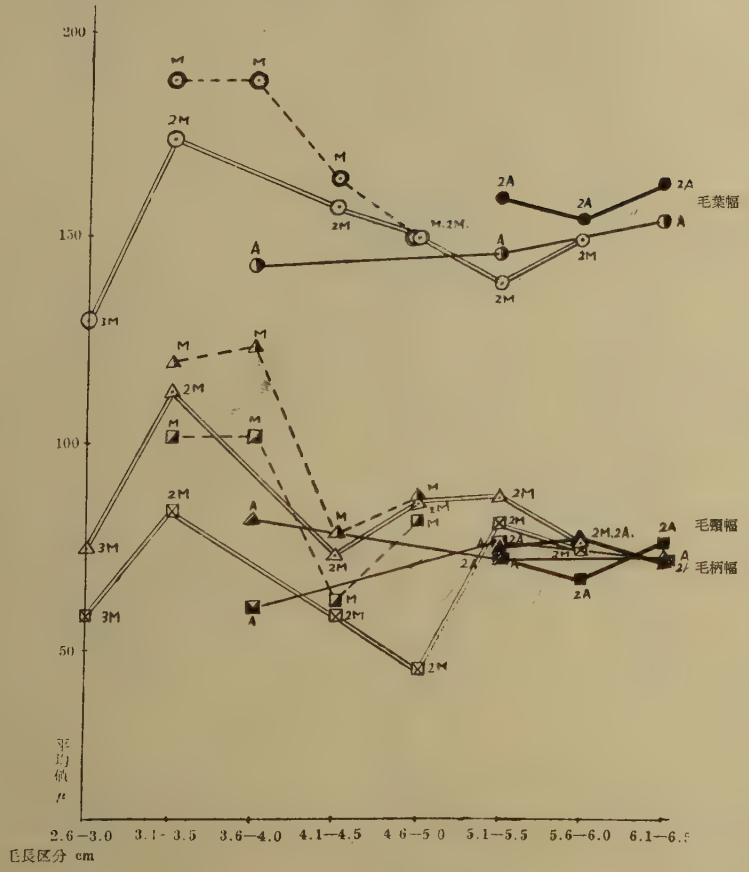


第29表　毛長の区分による柄幅，頸幅，葉幅の調査表

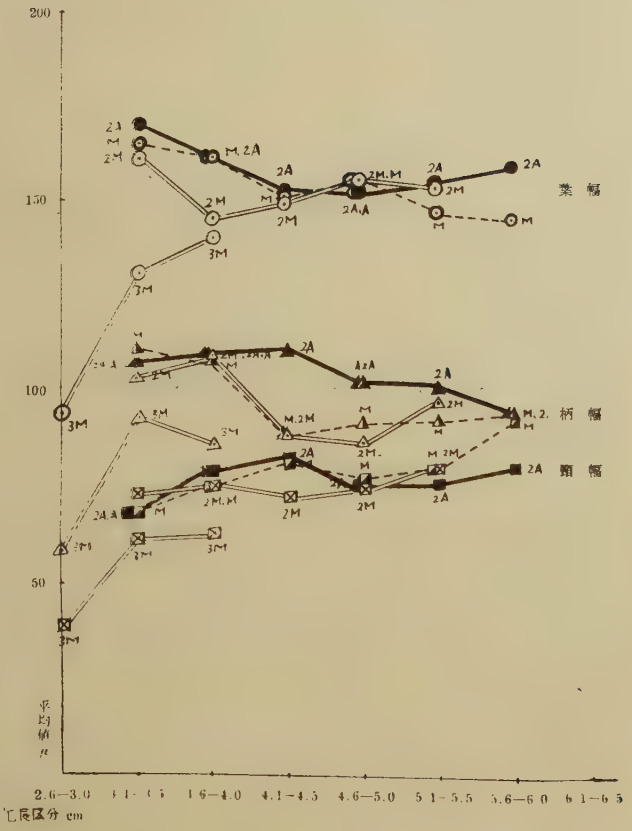
Animal no. N. A　背毛

| 毛長 cm | 球幅 μ | 根幅 μ | 毛柄 柄幅 (a) μ | 毛柄 髄幅 (c) μ | 毛頸 頸幅 (a) μ | 毛頸 髄幅 (c) μ | 毛葉 葉幅 (a) μ | 毛葉 髄幅 (c) μ | 明度 明 | 明度 暗 | 測定数 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.6～8 | | 63.9 | 119.7 | | 108.9 | | 157.5 | | | 2A | 2 |
| 7.1～7.5 | | 65.7 | 119.7 | | 108.9 | | 168.3 | | | 2A | 6 |
| 6.6～7.0 | | 63.9 | 121.5 | | 107.1 | | 166.5 | | | 2A | 19 |
| 6.1～6.5 | | 67.5 | 123.3 | | 107.1 | | 175.5 | | | 2A | 21 |
| 5.6～6.0 | | 65.7 | 121.5 | | 103.5 | | 166.5 | | M | | 4 |
| | | 63.9 | 114.3 | | 98.1 | | 168.3 | | | 2A | 8 |
| 5.1～5.5 | | 62.1 | 103.5 | | 92.7 | | 175.5 | | M | | 1 |
| | | 63.9 | 123.3 | | 94.5 | | 173.7 | | 2M | | 11 |
| | | 67.5 | 128.7 | | 101.7 | | 171.9 | | | 2A | 5 |
| | | 58.5 | 117.9 | | 94.5 | | 175.5 | | 3M | | 7 |
| 4.6～5.0 | | 63.9 | 119.7 | | 96.3 | | 177.3 | | 2M | | 10 |
| | | 56.7 | 126.8 | | 99.9 | | 170.1 | | | 2A | 2 |
| | | 69.3 | 128.7 | | 99.9 | | 173.7 | | M | | 4 |
| | | 67.5 | 125.1 | | 96.3 | | 179.1 | | 3M | | 15 |
| 4.1～4.5 | | 56.7 | 123.3 | | 96.3 | | 175.5 | | 3M | | 20 |
| 3.6～4.0 | | 62.1 | 117.9 | | 85.5 | | 173.7 | | 3M | | 20 |
| 3.1～3.5 | | 53.1 | 101.7 | | 76.3 | | 153.9 | | 3M | | 31 |
| 2.6～3.0 | | 38.7 | 72.9 | | 45.9 | | 105.3 | | 3M | | 30 |
| 2.1～2.5 | | | | | | | | | | | |
| | | 9.9 | 13.5～15.3 | | 11.7～13.5 | | 15.3～17.1 | | | 2A | |
| | | 11.7 | 13.5～17.1 | | 11.7～15.3 | | 13.5～22.5 | | M | A | |
| | | 13.5 | 18.9～22.5 | | 15.3～17.1 | | 20.7～27.9 | | M- | A- | |
| | | | | | | | | | 2M | 2A | 8 |
| | | 15.3 | 20.7～22.5 | | 17.1 | | 24.3 | | 3M | | |
| | | 17.1 | 22.5～29.7 | | 17.1～18.9 | | 26.1～35.1 | | 3M | | |
| | | 18.9 | 26.1 | | 18.9 | | 26.1～35.1 | | 3M | | |
| | | 20.7 | 26.1～27.9 | | 20.7 | | 26.1～35.1 | | 3M | | |
| | | 24.3 | 35.1 | | 26.1 | | 38.7 | | 3M | | |

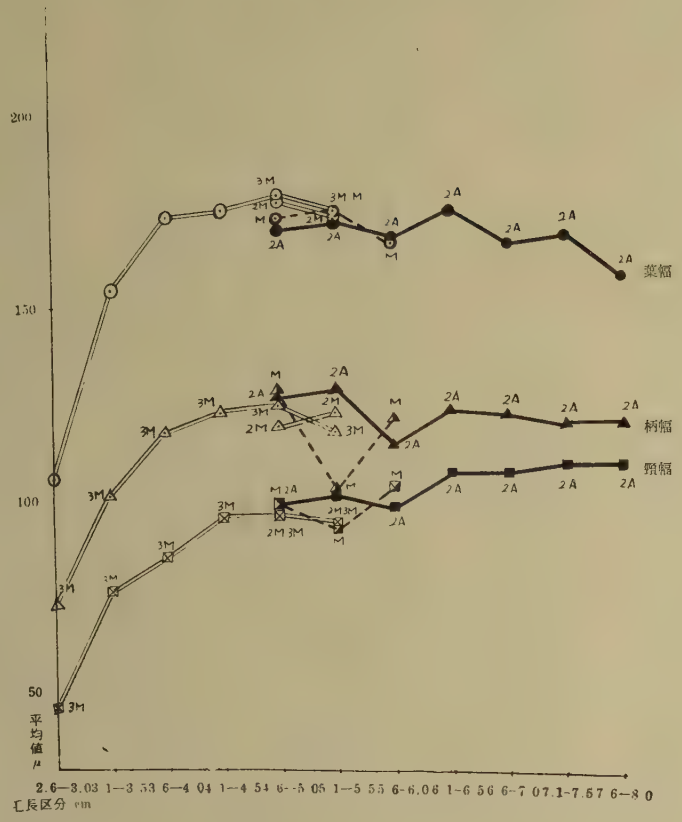
第22図　毛長と柄幅，頸幅，葉幅の関係図（KN. 4の背毛）



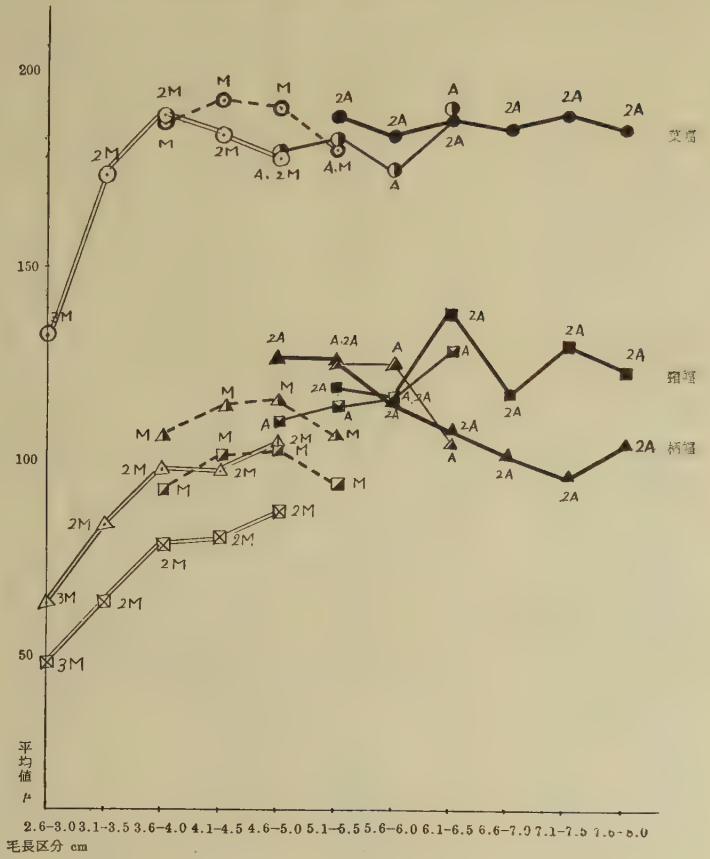

第23図　毛長と柄幅，頸幅，葉幅の関係図（KN V—8背毛）



第24図　毛長と柄幅，頸幅，葉幅の関係図（N. A 背毛）




第25図　毛長と柄幅，頸幅，葉幅の関係図（N. B. 背毛）



3. 髄質，皮質，色素の関係

L. A. HAUSMANN[19] は霊長類の毛の髄質を研究して次の5つの型に区分している．1. 欠除型（absent）2. 不連続型（discontinuous），3. 連続，不連続中間型（intermediate），4. 連続型（continuous），5. 痕跡型（fragmental），又この順序に毛の直径も肥大しているとした．この研究で猿類は背部の下毛（under hair or fur）について，人類は毛髪について調査し比較している．

筆者は Nutria 1種類について被毛の最小下毛から最大上毛までを，その髄質と皮質と色素との関係について研究し次のような結果をえた．

(1) 採毛繊維及びL切片による観察毛，繊維の検鏡による観察． Nutria の下毛の髄質は，毛根では痕跡として所々に点在し，毛柄部では定形的に気室を仕切り，連続して毛頸部に及び，毛葉部では不定形に継続して，毛芒部には欠けている．

小型下毛の髄質を低倍率（100—150倍）で検鏡すると，円形，楕円形，長方形の気室を仕切つて破線状に毛の略々中心を走っている（写 26，32，85，88 図）．

円形で約 4—5 $\mu$ の気室は小型な腹部下毛の内でも稀れであるが，この場合黒色部は比較的長くて約 6 $\mu$ で，数珠型に連っている．

次に背毛，腹毛で最も一般的な，気室が楕円形，長方形の場合では（幅3—4 $\mu$，長約 6 $\mu$）それと略々等幅，等長の黒色部で仕切られているので，ちょうど鉄道の線路記号のようである．これ等を高倍率（400—1,000倍）で検鏡すると，毛の尖端に面した郭線に黒褐色の色素顆粒が集結して後方が空虚である．この色素の集結箇所は前方が濃く後方に淡く，顆粒を欠いて空白になり気室との間に境界を認めえない（写 26 図）．又この気室の後部と次の色素帯との中間には気室に面して凹形の郭線が引かれ，その色素帯の前端とで色素顆粒のない凹凸レンズ型（meniscus 型）の部分を表わしている．これ等が破線状に連続している（写 26，32図）．

阿部[2]（1920）は毛の髄質の気室が細胞内に生じた空虚な部分から成るとして，詳細な観察を述べて，TOLDT や STÖHR 等の細胞間隙説に応えている．筆者の Nutria の小型下毛でも上述のように細胞形質はほとんど毛の尖端側に退き集結して後方に空虚な部分を留めたものといえる．

L 切片で観察の場合（下毛）． 次に背部の L 切片から，これ等小型下毛の毛包を検鏡して髄質の成り立ちから考察すると，毛乳頭を取り巻く球の頂点附近の毛母基から分裂して送り出される（GIROVD and LEBLOND[16] 1951）黒褐色の色素顆粒を多量に含んだ細胞はその外側の毛母基に由来した皮質の細胞層に周囲から包囲されている．孔道の入口にかかるまでの毛球の上部では色素顆粒が核を被うて不規則に並んでいる（写 94，95，96図）．細胞は孔道の入口，すなわち毛髄質の基部ではジグザグに重なり合っているが，孔道に入ると色素顆粒が前方に移動し，核は後方に留まって，細胞は孔道に満ちて長楕円形になる（写 95 図）．後方から続いて押し上って来る一連の細胞の核は次第に形が縮小して遂に上方では染着性を失なって消失し，色素顆粒の集結部を黒褐色に残して，後方に核の占めていた部分を空虚な気室として留めることになる．

以上はヘマトキシリン，エオジンによる核形質の染着から明らかに追求しうる．即ち図のように色素顆粒のすき間を透して核が見えるが，漸次核は後方に現われて1部分は顆粒群に被われているが徐々に食いちがってゆき，後方に接触してとどまっている（写 95，96 図）．

角化の初期の内は，核は1個で皮質の挟む孔道に詰って髄幅を占めているが，上方に移るに従って染着した核形質は小型になり，上方の濃色部に退いてゆき遂に消失して空虚になっている．

皮質細胞は毛球の上位からすでに縦に長い楕円形を示して紡錘形に角化する趨勢にあり，従って細胞内の色素は上位に押し上るとともに，縦に長い色素顆粒塊乃至は顆粒群を形成している．

小型下毛の皮質では，毛根部の髄質痕跡の周囲に色素は微量で，毛柄部には均一に散在して灰褐色の淡調で，常に髄質の色素帯の濃度には及ばない．

毛頸部，毛葉部では濃褐色の皮質の色素に被われるので，不定形に断続している髄質の有色帯は不明瞭である．従って下毛は一般に下半分は淡色で上半分は濃色の色調を表わしている．

腹部の小型下毛の内にはしばしば髄質の痕跡が所々にあって色素顆粒が微量に集まっており，それを中心に附近の皮質にも色素が点在していて淡色で，毛の全体が凝膠色の白色に近いものがある．又髄質には色素があるが皮質には欠いている場合があり，常に腹部下毛は背部下毛に比べて淡色である．

更に背，腹毛ともに髄質と気室は明らかにありながら，全く色素顆粒を欠いていて，凝膠色又は乳濁色で，albino と同様の白毛もある．この種の下毛が総毛量の10%に近い個体もある．

個々の下毛を検鏡するにはグリセリンで透視するので髄質の色調の方が濃厚であるが，上毛を抜き取って下毛のみ残して，毛皮に利用する場合の外観的な色調はこの皮質の色素の濃淡で決まるようである．従って毛の色調は皮質が瀰漫性淡黄色の基調色に一面に褐色，黒褐色顆粒が散在するときは煉瓦色，チョコレート色に，凝膠色，乳褐色の基調色に褐色，黒褐色顆粒では灰褐色，灰色の色調になる．

L 切片で分岐毛の観察，　柄幅が約20—25 $\mu$，髄幅が約 10—15 $\mu$ の分岐毛の毛髄部は気室が毛幅とともに横に拡張して，楕円形の長径を立てて並べた形になり，遂には円味を失なって長方形に変り，小型下毛の場合の隔線と長方形の色素帯の位置に，前半が淡色で後半が濃色な分厚い隔壁を表わして，小型下毛の場合と異なり明らかに気室を仕切って，梯子形に連続している．この前半の淡色部或いは無色部は前方の細胞形質の1部で，後半の濃色部は後方の細胞形質の色素顆粒を含んだ部分である（写 26，53 図）．

これ等の髄質は前記のように髄幅（長幅）に比べて壁と気室の長さは短かいが（8—12 $\mu$），細胞1個の占める空間としては広過ぎるようである．尚小型下毛の場合と同様に黒褐色顆粒は前方に移動して核が後方に留っているが，未だ縮小しないで孔道の入口附近にある核も，小型下毛のように孔道内をふさいでいない．がいして髄質の細胞が一列に並んでいる場合は，細胞の色素が先に移動して核が後方に留って気室の基となるようである（写 96，98 図）．

又分岐上毛では，毛柄の柄幅は略々 25 $\mu$ まで長径，短径の差が明らかに現われていない，髄質の隔壁に破損の個所のあるものは，隔壁と気室の周縁の色素顆粒の分布は平均している．

下毛のように色素が一方に偏在して色素帯とか，隔壁の両側が濃淡の染め分けになっていない．これは髄細胞形質中の色素顆粒が細胞膜の周囲に平均に退いて分散した結果，前方の細胞形質の一部と後方の細胞形質の一部からなっている仕切りの隔壁も濃淡の染め分けなく，

均一に着色していることになる.

註. 分岐毛まではL切片中の繊維と採毛繊維のグリセリン浸漬の観察とは同様であるが, 毛柄が楕円形に変ると, 前者は側幅, 後者は正幅面の観察となる.

小型第2上毛（採毛繊維の検境）, 柄幅が増して約25—30μとなり, 分岐上毛から小型の第2上毛に移行すると, 横断面が楕円的に変り同時に髄質は約13—15μとなって, 所々に隔壁の破損が現われて来る. これは遂に1個の細胞ではこの髄幅をふさぎえなくなった徴であろう.

この場合隔壁の厚さとその間隔（気室）は共に約3—4μで, 小型下毛の長方形の色素帯と空白部（気室）の略々半ばである.

柄幅が増して約40—50μの場合は, 髄質が約20—25μで皮質に附着する隔壁とその間隔は, 前記と略々同様で4—5μであるが, 両側の皮質から突き出している隔壁の端は互いに向う側にとどかない. 最早1個の隔壁が中途から破損したもののように相対していないで, 別個のものが両側から突き出して互いに交合し, 又は互いに喰い違っているようで, 細胞の配置は乱れ始めて, 1列からジグザグに変っている（写53, 98図）.

大型第2上毛から第1上毛（採毛繊維検鏡）, 更に柄幅が拡張して来ると, 両側の皮質に附着する部分の髄質の細胞形質とその間隔は, 小型毛と略々同様で3—5μで, やや正しく櫛の歯状に並んでいる. その中間の髄質の細胞形質は不規則に続いて波型になり, 上下に接近して両側の皮質の間に（柄幅面に）莚の表のような紋様を表わしている（写100図）.

以上は完全に角化した棍状毛の毛柄部をグリセリン浸漬で柄幅面から検鏡した平面的な観察であるが, これをL切片で検鏡すると, 毛球部から脂腺の上位を経て表皮面まで, 即ち毛包部から毛管部までの, 毛が発育して完全に角化する変化過程を毛柄の側幅面から平面的に観察することになる. そのとき毛球の下端から脂腺の下位までの下方約$\frac{2}{3}$以内は髄質及び皮質の核形質がヘマトキシリン, エオジンに染着している. 毛小皮の核は判明しないが毛球上位の小部位で不染性になる（写99—102図）.

次にその形態的な変化を述べると, 毛球の上位から髄質の孔道の入口附近の細胞では, 細胞形質が細胞膜の周縁に色素顆粒とともに後退して内部は空虚であるが, その細胞形質の大部分が前方（毛尖端の方向）の周縁に集結している. この濃色部の後部に染着した核形質が附着して細胞の略々中央に位置している. これ等の細胞の周囲の一面は皮質に他の面は互いに同形の髄質細胞に接着して, 不規則に略々2列に並んでいる. 漸次上方に移ると細胞形質の角化が進み細長く緊張して, 梁（はり）状に連絡している. 核形質は前方の濃色部に附着しているが漸次上方では縮小して遂いに消失している.

更に毛柄が大型になり, 長径と短径の差が著しくなると, 色素顆粒は細胞の周縁に略々平均に後退して中央に空白部を生じて, 毛包の下位では上記のように細胞は接着して連絡しているが, 角化するに従って核形質が上方の細胞形質の壁に附着し, 梁状に緊張していた細胞形質の多くは中間が欠損してジグザグに通気孔を生じ, 最早隔壁で封じられた気室ではなくなる. この切断された角化部分では, 染着した核形質は皮質の側面に附着する個々の細胞形質の略々中央に附着しているが, 上方では消失する.

大小の上毛を通じて, 毛柄部, 毛葉部の髄質の側幅は約10—20μの間にあるので, 髄質の側幅面からの観察では, 細胞は略々1列乃至は2列に連続している（写102, 103, 98, 106, 108, 113図）.

小型上毛では細胞形質は連絡を保って破損は少ないが，大型上毛では両側の皮質に附着して2分されている場合が多い．前述のように髄質の細胞形質が角化縮小した場合には2個分の細胞形質では髄側幅を充分に連絡しえないことになる．

(2) R切片による観察

R切片では他の毛群と比較によってその発育現状を推測することは許されるが，個々の毛についてそれを追求するには連続切片によらねばならない．尚毛の成育経過の観察は毛球下端から表面に及ぶ略々2.5　3.0 mm の間の連続R切片によるのであるから，毛根の上位或いは毛芒部のように短かい範囲で形態が急激に変化する部位は別として，毛の大部分を占める他の部位では，この短かい区間で大きい差異は無いので毛球部から脂腺上位までの毛包部を同一部位の発育過程とみて比較することが可能である．

下毛の観察

小型下毛のR切片は柄幅約10—15 $\mu$ の円形に髄幅約3 $\mu$ の毛髄部が略々中央に位置して濃色な黒褐色を表わしている．R切片はいづれも約20—40 $\mu$ の厚さがあるので小型下毛の場合は黒褐色から黒色で，数個の有色帯や（色素顆粒のある髄質の実質）気室がこの間に挟まれており，これを上方より検鏡するので気室のみを含む無色の場合は起らない．

分岐毛の観察

分岐毛の毛柄では長径，短径の差異が生じて髄質の断面積がやや広くなり，髄質とその附着する周縁の皮質部に色素は平均に分散して色調は淡色になる．色素を含んだ髄質が長径の両端に引き離されて，中間が欠損し，その色調は均一で濃淡はない．又同じ焦点下で両者が明瞭に現われるので1個の細胞形質の分離したものと認めうる．やや柄幅を増して来ると表皮面に近い角化した部分では髄質は楕円形の両端に近く細胞形質が色素顆粒を集積して偏っていて，両端に焦点が同時に合はないので，両者の間に上下の間隔があることが判る．尚毛包の下部では核形質が染着されるのでその関係がさらに明瞭である．最早染着した核形質は下毛のように髄質の中心に1列に並んでいないで，これを結ぶ線はジグザグになるが，まだ髄質は1列の細胞から成立している（写98図）．

小型上毛の観察

分岐上毛の域を脱して，小型第2上毛の毛包の下部では髄質の楕円形の両端は染着した核形質を中心とする細胞形質が同じ焦点下に2個現われて，これ等が同様に鮮明な場合（写111図），一方がやや不鮮明な場合（写115図）或いは又同じ焦点下に3個現われるが1個が不鮮明な場合，などの変異が現われて，細胞の列が1列から2列に変化する経過を物語っている．このとき毛球の上位では髄質は略々円形に近く面積も広いので3個以上の細胞が同じ焦点に見出されることになる．これ等は髄質の側幅が約10—15 $\mu$ までの小型上毛の場合でL切片で観ると髄質の細胞形質は縦に1列になっている（写96，98図）．

大型上毛の観察

大型上毛の完全に角化した毛柄をR切片で観察すると，髄質の側幅に2個，髄幅に数個乃至10数個の細胞形質が略々2列に並んで楕円を埋めているが，L切片で観察した場合はジグザグの通気路を表わしているので，上方から焦点を上下して観ると，長径（a）に沿う細胞形質の2列の間に僅かの間隙が発見される場合，又は両側の形質の一部は連絡を保って一平面内にあり，他は欠損しているが下部の形質が重なって一平面を仮装している場合，などがある．これ等角化部分の観察では，平面的には濃淡のモザイック型の紋様を表わして，個々

の細胞形質の判別はほとんど出来ない（写101図）.

毛球の上位では毛の断面は長径と短径の差が少なく，円形に近い．従って髄質も同様略々円形で皮質細胞も髄質細胞もその核の形態はほとんど差異がなく，又髄質細胞は雑然と並んでいる．細胞形質に含まれている色素顆粒は細胞の接触面に比較的多く集っていて微かに紋様を表わしているが，皮質，髄質の区別なく薄墨色の紗で被うたように一面に彩っていて，髄質の核形質の染着が皮質に比べて僅かに濃色である．さらに髄質の孔道の入口附近から髄側幅が短縮して細胞が2個以上は並びえなくなるので，長径側に長く略々2列に並んで詰まるようである．これ等の細胞は染着した円形の核形質を中心にして黒褐色顆粒の多くは細胞周縁に退いて，細胞との接触線を明瞭に連絡して蜂巣状に並んでいる（写106，108，113図）.

さらに上位に移り角化が進めば核形質の染着はなく，細胞形質も角化により歪を生じて来るので，蜂巣状に釣り合っていた細胞の接触縁が乱れて色素の配置は不定形になり，上述のようにモザイック型の濃淡を表わす.

さらに各部位の横断面積から観察を附け加えると，柄幅は上，下位とも略々同様であるが（上部がやや大きい場合もある），側副は下位がはるかに大きいので，まだ角化しない時の横断面積は広いことになる．髄幅は上位が大きく，髄側幅は上，下位とも略々同様であるので髄質の横断面積は，下位の未だ角化しない部分が狭いことになる．従って漸次上位にうつるとともに髄質の占める面積は広くなるが，逆に細胞形質は角化収縮するので自から空白が生じて，通気路，空洞の素因となる.

更に皮質部の占める面積は下位が広く，又核形質の大きいことから細胞形質の角化不充分が知られる．とくに下位において毛球の上部の断面の面積は，いづれの毛幹部の断面積よりやや広くて円形である．又細胞の配列は雑然としていて，その数も多い．ここでは皮質細胞の核も髄質の核と同様で判別は難しいほどである．尚完全に角化した部分は，未だ発育中の青毛の場合とすでに成毛になって長期間棍状毛の状態で皮面に留っているものとを比較することになるので，髄質部の破損は各種切片の場合の皮面に接する角化部よりも，成毛のグリセリン検鏡の場合に比較的多い．これは時間の経過に従って過剰乾燥，皮脂の不足，又は外力等によって受ける被害の結果と考えられる.

以上は凡て上毛の毛柄部について述べたのであるが，次に毛の前半を占める毛葉部について考察する.

毛葉部の観察

上毛の毛葉部は分岐毛，小型第2上毛の一部のものは，葉幅が狭く，葉幅と葉側幅との差異が少ないので毛柄部の場合とよく似た形態である．しかしこの範囲は甚だ狭くて，毛葉の多くは毛柄の場合と異なり，側幅は毛柄部の側幅に比べて狭く，葉幅がはるかに広いので細胞形質の角化の形態も自から異なっているようである.

前述のように毛柄部の髄質の各面の観察結果によると，細胞形質の角化物は扁平に傾斜している．これは軟質の時期にやや平均した張力によって緊張されたことが考えられる.

毛葉部では髄側幅が狭く，髄幅が極端に広いので張力が髄幅に強く加わり下記のようにピラミッド型に切断されていると考えられる.

大型上毛の内で，黒褐色の濃色な暗葉毛の第1上毛の場合

毛葉部の観察は困難であるが，一般の明葉上毛では完全に成長した棍状毛のグリセリン浸

漬，及び S 切片で脂腺上方の完全に角化した部分を検鏡すると，この場合は正幅面を観察することになるので，毛葉面は不規則な蓮の表の様な紋様を表わしている．即ち S 切片では，毛包の下位は鱗形に無色の線で個々の細胞に区画されて細胞の周縁に黄色顆粒が多量にあり，中央に染着した核形質があり，上方に移るに従って細胞形質が縮小して無色の画線の部分は拡大する（写99図）．

遂に完全に角化した上方部では核形質の染着は消失して細胞形質は収縮し，断続的に鮮明に浮き出して来る．間隔は拡大して縮小した細胞形質と略々幅が等しくなる．

尚 L 切片から発育中の観察では，前述の毛柄部の場合のように毛包の下位で，まだ細胞形質の角化の進まないうちは互いに接着しているが漸次硬化するに従って個々に離れて皮質の側面に附着して相対している．この時染着した核形質は細胞の中心にあって，暗葉の場合は黒褐色顆粒が細胞形質全面を被い核形質は明瞭に透視出来ない．又明葉の場合は黄色の瀰漫性色素の基調色に黄色顆粒が内在しているので，毛柄部の L 切片の観察のように個々の細胞の内部に空虚な個所が生じて，この部分が切断されて他の細胞形質と合併するかのような，詳細な観察は困難であるが，がいして角化初期に多量の細胞形質が細胞内で前方（毛の尖端に向って）に移動して収縮するようである．毛包下位では毛柄部と同様に両側の皮質に挟まれて略々2列に接着しているが，やや上位では皮質側にピラミッド型に底面を附着させ，中央に核形質を配して，鋸歯状に両側から噛み合っている（写102，103図），その上位では分離してジグザグ型の通気路となり，さらに上位の充分に角化した部分では細胞形質は鈍端を表わし先端の間隔が大きく開き空洞型になる．

R 切片では前記の毛柄部と同様，角化の進まない毛包の下位では髄質の孔道内で長径に沿って長く略々2列に蜂巣型に細胞は接着して中央に核形質が染着して色素顆粒は黄色，褐色の別なく，その周囲の細胞の接着部に多く退いて細胞の境界を明瞭に表わしていることは大型の毛柄部と同様である（写108，113図）．

Hausmann[19]（1924）は髄質の欠除したものから，個々に独立した気室の連鎖状のもの，気室の隔壁が破損してジグザグな通気路型のもの，隔壁が全く欠除して空洞型のもの，所々に空洞の断続するもの等，5種に分類してその順序に毛幅は拡張しているとしたが Nutria の場合は欠除型のものはなく，腹毛の最小下毛に稀れに Hausmann の不連続型の髄質を断続的に留めるものがある．一般の下毛の場合は気室の連鎖する Hausmann の不連続型（discontinuous）のものであり，分岐毛から小型上毛では隔壁が破損してジグザグの通気路型の髄質に転移を示しており，上毛ではジグザグの通気路型の髄質となっているが，Hausmann の intermediate 型や，continuous 型ではない，更に fragmetal 型の場合はない．

## 摘　要

1）下毛の毛髄は気室と髄質と交互に並んで連鎖状である．

2）毛髄は髄質細胞が一列に連続している場合のみ完全に閉じられた個々の気室を形成している．

3）この気室は髄質の核形質が占めていた空間であって，従って気室は細胞内に形成されている．

4）上毛の毛髄は角化した細胞形質が皮質の孔道壁に略々等間隔に附着して互いに周縁か

ら内部に接着し，対面し，又は交さしてその間にジグザグの通気洞を形成している．

5) 色素顆粒は下毛の場合は気室を隔てる髄質に多量に存在しているが，毛幅の広いもの程，皮質と髄質の色素は平均に分散している．

6) 毛小皮は皮質と髄質より早期に核形質の染着性が消失することにより，毛小皮の細胞形質の角化は皮質，髄質より早いようである．皮質と髄質の核形質の染着性は略々同時に失われるようである（写102図）．

4. 毛小皮と皮質，髄質との関係

HAUSMANN (1924) は毛小皮 (cuticular scales) を coronal scales と imbricate scales の2つに分類し，上坂等 (1950) はこれに冠状 (cornal) と瓦列状(imbricate) の邦語をあてている．

HAUSMANN は冠状毛小皮は1個で毛幹を取り巻いているが，瓦列状毛小皮は1個で取り巻きえない場合としている．又瓦列状毛小皮を卵形 (ovate)，鋭尖形 (acuminate)，長形 (elongate)，鈍鋸歯形 (crenate)，平縁形 (flattened) の5種に，冠状毛小皮を単純冠状 (simple scales)，鋸歯状 (serrate scales)，歯牙状 (dentate scales) の3種に分類している．

MÜLLER (1939) は冠状毛小皮の鋸歯状の例をあげて数個の鱗片の融合したものとしている．

尚 HAUSMANN は毛小皮の重合基部から末梢部の遊離縁までの幅 (F) と毛の断面の直径 (D) との比率 ($\frac{F}{D}$) を scale index (毛小皮係数) として表わし，彼は霊長類の被毛について研究し毛繊維の粗大なもの程又毛小皮の紋様の複雑なもの程，scale index は小さいとしている．

又岡島，池田等(1955)はアンゴラ兎毛の捲縮 (crimps) とこの毛小皮 (cuticular scales) の関係について述べている．堀尾，近藤等 (1953) は羊毛の捲縮の機構について研究し，E. MERCER (1953) は更にこれを化学的に解明している．

筆者は Nutria の被毛で，毛小皮を中心に皮質，髄質との間に次のような関係のあることを認めえた．

下毛の捲縮の観察

柄幅が 10 $\mu$ 前後 (約 9.9—13.5 $\mu$) の小型下毛は一主として腹部下毛である一毛長約 1.5 cm に約20個の捲縮 (crimps) がある．毛柄をグリセリン浸漬の検鏡では（写26, 28, 86図）彎曲部は髄質を中心として皮質が彎曲外面（旋回の外側）厚く，彎曲内面の側に薄い．外側の皮質は内側より過剰に成長している．毛小皮は sump の標本によれば常に毛先に向って彎曲内面側に傾斜し，冠状の毛小皮の連りは縄紋型を表わしている．毛小皮の1個を融合し又は接着した円筒形の冠状として，冠の下底の重合部を作図（第57図）のような形状とみなせば彎曲内面側の毛小皮の幅は彎曲外面に面した幅よりやや長く，順次に前方に張り出しており，外側の皮質の圧力に耐えて極度に毛幹が屈曲するのを支えているかのようである．尚毛繊維が毛根の上位から個々に略々左右両旋回の2様の場合あり，又1個の繊維が部分的に左右両様に旋回する場合あり，とくに不定形な crimps を持っている大型下毛の場合には小部分的に左右両様旋回をする傾向がある．

例えば一左旋回する繊維の左側の彎曲から右側の彎曲に毛小皮の紋様をたどってゆくと，右側に移る渡りの部分の初めは皮質の薄い部分が上面になり，厚い部分が下面に廻るので，

髄質は繊維の中央を走っているように透視される．毛小皮は毛先に向って凸形縁が重なり合っているが漸次薄い皮質が左によじれ（内側）て，厚い皮質の部分が右に廻る（外側）につれて毛小皮の凹形縁重合部は右に移って繊維の彎曲の山は右側に移り，皮質の厚い部分は全く外側となる．従って髄質は内側に薄く，外側に厚く皮質を両分している．この際毛小皮は常に毛先に向って彎曲外面から彎曲面に傾斜している．

次に右側の彎曲から左側の彎曲をたどってゆくと，渡りの部分の初めは毛先に向かって凹形縁が重なり合っていて，皮質の厚い部分が上面に表われ，薄い部分は下面に隠れているが，漸次上面の厚い皮質の部分が左に廻わって外側に，薄い部分が右に（内側）よじれるに従って毛小皮の凸形縁の重合部は右に移って彎曲の山は左側に代り皮質の厚い部分は全く外側となる．以上は左旋回する繊維であるが右旋回の場合は全く逆である（但し Sump の標本は実物の全く反対の面を写している）．

又毛根部，毛芒部は中心に髄質を欠いて皮質のみの均等質で，毛小皮は縄紋型でなく不整な長円筒形の冠状である（写29図）．毛葉部は下毛，分岐毛を通じて一般に縄紋型の配列である（写25，35図）．以上は柄幅が 10 $\mu$ 前後の小型下毛で主として腹部の下毛にあてはまる．更に柄幅が約 15 $\mu$ までの中型下毛の 1 部は，毛長 1.5—2.0 cm に約10個の捲縮があり，上述の法則に従っているようである．これ等は多く背部下毛にあてはまる．

約 15 $\mu$ 以上の大型下毛及び分岐毛では毛柄の左右，同側に毛小皮の凸縁部或いは凹縁部が数個重なり合って交互に配置されて，互いに歪を取り戻すかのように表われており，又同側に凹凸の部分が交互に重なり合って，常に歪の均衡を保っているかのようである．

次第に毛幹は彎曲を減じて crimps とよばれる程の定形的なものでなくなる．この場合髄質は略中心を走っていて，皮質は髄質を中心に四方に略々均等の幅を保っている．気室の仕切りは梯子型である．毛葉部のみは葉幅をやや増して縄紋は一層密に（2—3$\mu$）なり，一方に彎曲している．髄質は明らかに彎曲内面に偏っている．以上のように下毛及び分岐毛を含む小型毛は一方的の転倒からまぬがれるような構造を持っている．

毛小皮の観察

背毛の場合（sump の標本による測定例）

1） 第1上毛，暗葉毛，毛長 7.5—8.0 cm，柄幅 134.1 $\mu$，葉幅 161.1 $\mu$，毛柄の毛小皮の幅は約 20 $\mu$，その長さは約 30—50 $\mu$ である．

毛葉の毛小皮の幅は約 10 $\mu$，その長さは約 50 $\mu$ である．毛小皮の厚さは約 2 $\mu$ であるが，2—3 個重なっていることは，R切片観察の毛球上部の断面の細胞により明瞭である（写108図）．

毛柄，毛葉とも毛小皮は瓦列状の配置である（写60，62図）．

2） 第2上毛，明葉毛，明度 3 M，毛長 3.6—4.0 cm，柄幅は 119.7 $\mu$，葉幅は 157.5 $\mu$，毛柄の毛小皮の幅は約 10 $\mu$ で，その長さは約 50 $\mu$ である．毛柄，毛葉ともに瓦列状配置である．

3） 第2上毛，明葉毛，明度 3 M，毛長 2.6—3.0 cm，柄幅は 72.9 $\mu$，葉幅は 105.0 $\mu$，毛柄の毛小皮は約 10 $\mu$，長さは約 30—50 $\mu$ である．毛柄，毛葉ともに毛小皮は瓦列状配置である（写44，45，46，47図）．

4） 第2上毛，明葉毛，明度 3 M，毛長2.6—3.0 cm，柄幅は 53.1 $\mu$，葉幅は 58.5 $\mu$，毛柄，毛葉とも毛小皮は瓦列状配置である（写54，55，58，59図）．

5) 第二上毛，明葉毛，明度3M，毛長2.1—2.5 cm，柄幅は31.5 μ，葉幅は36.9 μ，毛小皮は毛柄で幅5—10 μ，毛葉で約8 μ，毛小皮は冠側の所々に継目，合せ目が生じて上縁は鈍鋸歯状になり冠状より瓦列状の配置に移らんとしている（写56図）．

6) 分岐上毛，明葉毛，明度毛M，毛長1.6—2.0 cm，柄幅は18.9 μ，葉幅は22.5 μ，毛小皮は毛柄で幅約12 μ，毛葉で幅約5 -6 μ，毛柄では略々単純冠状で，毛葉では縄紋型の冠状である．冠側に合せ目を増加している．

7) 分岐下毛，暗葉毛，毛長1.6—2.0 cm，柄幅は18.9 μ，葉幅は17.1 μ，毛小皮は毛柄で幅約10 μ，毛葉で約3—6 μ，毛柄の毛小皮は単純冠状と縄紋型冠状の混合型で不定形に捲縮している．毛葉は縄紋型冠状である．

8) 下毛，暗葉毛，毛長1.5—2.0 cm，柄幅9.9—15.3 μ，毛小皮の幅は約6—10 μ でともに縄紋型冠状であるが，毛葉はがいして毛小皮の幅は狭い．最小下毛の毛小皮は冠側に合せ目のない冠状のものが多い．

部位によりやや肥大する場合には合せ目を生じている（写84図）．冠側の合せ目は凸縁側になく，凹縁側にある（写84図×印）．即ち毛の捲縮の場合はがいして螺旋の外側に当る部分に合せ目がある．

毛葉は葉幅約25 μ までは縄紋型冠状の小毛皮で被われ彎曲外側の凹縁部に合せ目を生じているが，略々30 μ では冠状と瓦列状の中間型を示していて，最早毛葉の断面は円形ではなくて，不整形な楕円形で，葉幅はその長径で示すことになる．この際縄紋型で冠側に継目を生じて毛葉を取り巻く毛小皮は複数形の傾向を表わして来る．葉幅が約35 μ では縄紋型の紋様はなく単純な瓦列状を表わして，断面はやや正しい楕円を示して縄紋型の毛葉のように極端に彎曲しないで，スライドグラスの上で平らに検鏡できる（写52，56図）．

腹毛の場合，(sump 標本による測定)．

1) 第2上毛，明葉毛，毛長3.6—4.0 cm，柄幅は83.7 μ，葉幅は155.7 μ，毛柄の毛小皮の幅は約6—8 μ，その長さは約35—45 μ，毛葉の毛小皮の幅は約5—12 μ，長さは45—50 μ である．毛柄，毛葉とも瓦列状の毛小皮である．

2) 第2上毛，明葉毛，毛長2.1—2.5 cm，柄幅は90.9 μ，葉幅は125.1 μ，毛柄の毛小皮の幅は約8 μ，長さは約53 μ，毛葉の毛小皮の幅は約3—17 μ，長さは20—40 μ である．毛柄，毛葉とも瓦列状である（写48，49，50，51図）．

3) 第2上毛，明葉毛，毛長1.6—2.0 cm，柄幅44.1 μ，葉幅は71.1 μ，毛柄の毛小皮の幅は約10 μ，長さは毛小皮1個で柄幅を満たすことは稀れで約40—50 μ で単純な瓦列状である．

4) 第2上毛，明葉毛，毛長1.6—2.0 cm，柄幅は26.1 μ，葉幅は49.5 μ，毛柄の毛小皮の幅は約5—6 μ，長さは冠側に継目，及び合せ目あり約50 μ で未だ冠状といえよう．毛葉の毛小皮の幅は約6—8 μ，長さは25—40 μ で単純な瓦列状である．

5) 分岐毛，明葉毛，暗葉毛，毛長1.0—1.5 cm，柄幅は17.1—18.9 μ，葉幅は20.7—22.5 μ，毛柄の毛小皮の幅は約8 μ，冠側に合せ目を生じ単冠状で捲縮はない．毛葉の毛小皮の幅は約5 μ，毛葉の彎曲外側の凹縁部に合せ目があるものあり縄紋型冠状である．

6) 下毛，毛長1.0—1.5 cm，柄幅は9.9—13.5 μ では，毛小皮は大部分が縄紋型冠状で所々に短い単純冠状の部分が混在している．定形的な緩やかな捲縮（crimps）があり，彎曲の山は1.5 cm 間に約20を数える．柄幅 15.3 μ では，捲縮は不定形となり毛小皮はやや

長い縄紋型冠状の配列の部分と，数個の毛小皮が境離縁を斜めに平行させて重合し，これが交互に冠側に現われている部分，又は冠側の高低側を交互に重ね合わせている部分などがある．又所々に冠側に合せ目が現われている．毛葉は葉幅 9.9 -17.1 μ では，毛小皮は幅 2—5 μ で大型のものはやや狭い．毛柄より細目の縄紋型冠状である．葉幅 22.5 μ では細目の縄紋は合せ目，継目あり複数形を示して瓦列状に入らんとしている．葉幅の約 20 μ が縄紋型の限界である．

毛の横断面の観察．

背毛の場合（ℝ切片による検鏡測定）．

毛柄（hair stalk）

1) 毛球の上位（未角化），a, b, 15.3 μ→脂腺位以上（角化）11.7 μ，では皮質の染着核形質 6 個，毛小皮の染着核形質 1 個髄質の染着核形質 1 個である．

2) 毛球上位 a, b, 24.3 μ→脂腺位以上 a, b, 20.7 μ，では焦点の移動により 2 個の毛小皮が対面している．又同じ焦点下に 2 個の場合もある．

3) 毛球上位，a, b 26.1 μ→脂腺位以上 a, b, 22.5 μ，では同じ焦点下に毛小皮の染着核形質 1 個あり．

4) 毛球上部，a=40.5 μ，b=35.1 μ，c=13.5 μ，d=9.9 μ→毛球上位，a=29.7 μ，b=26.1 μ，c=11.7 μ，d=8.1 μ→脂腺位以上（推定），a=26.1 μ 毛小皮の染着核形質が 2 個宛々焦点移動により交互に現われる．

5) 毛球上位，a=35.1 μ，b=29.7 μ，c=13.5 μ，d=11.7 μ，毛小皮核 2 個は明瞭，1 個は不明瞭であるが 3 個同焦点下あり．

6) 毛球上位，a=35.1 μ，b=26.1 μ，c=d=8.1 μ→脂腺位以上，a=26.1 μ，b=22.5μ，c=d=8.1 μ，毛小皮の染着核形原が同焦点下に 2 個現われる．

7) 毛球上位，a=47.7 μ，b=35.1 μ，c=d=26.1 μ，→脂腺位以上，a=35.1 μ，b=24.3 μ，c=17.1 μ，d=9.9 μ，毛小皮の染着核形質が同焦点下に 3 個現われる．

毛葉（hair blade），

1) 毛球の上位（未角化），a, b, 26.1 μ→脂腺位以上（角化）a, b, 22.5 μ では暗葉毛で皮質不明，毛小皮の染着核形質 1 個現われる．

2) 毛球の上位（未角化），a, b, 24.3 μ→脂腺位以上（角化）a, b, 20.7 μ，毛小皮核 1 個，暗葉毛で皮質不明．

腹毛の場合（ℝ切片による測定）．

毛柄（hair stalk）．

1) 毛球上位（未角化）a, b, 13.5 μ→脂腺位以上（角化）a, b, 9.9 μ．

毛球上位（未角化）a, b, 17.1 μ→脂腺位以上（角化）a, b, 13.5 μ．

この範囲では皮質の染着核形質は 5—7 個，毛小皮の核形質は 1 個で，又髄質の核形質は毛球上位では同じ焦点下に 2 個現われるが，1 個はとくに鮮明である．更に上位の部分では 1 個で，完全に 1 列に並んだ細胞の髄質である（写87, 89）．

2) 毛球上位 a=b=20.7 μ→脂腺位以上 a, b, 17.1 μ で，皮質の核質 7 個が 1 重に髄質を包囲している．毛小皮核形質 1 個である．

3) 毛球上位 a=b=20.7 μ→脂腺位以上 a, b, 17.1 μ．

毛球上位 a=b=24.3 μ→脂腺位以上 a, b, 20.7 μ．

この範囲では焦点の移動により毛小皮の核形質は毛の断面の円周上に対面している．髄質の核細胞は1個で1列である．

4) 毛球上位 a=b=24.3 μ→脂腺位以上 a=b=20.7 μ．これでは同じ焦点で2個の毛小皮の核形質がある場合もある．

5) 毛球上部 a=b=27.9 μ.

皮質の核形質は10 11個，毛小皮の核形質は不明，髄質の核は6個で，皮質の細胞は1重の環状に配置している．皮質細胞の数及び配列型，色素の量等で略大型下毛，分岐毛の毛柄と推定される．

6) 毛球上部 a=27 μ，b=29.7 μ，毛小皮の核形質は4個同一焦点下にあり．皮質細胞の核形質は明瞭でない，髄質の核形質は2個である．

毛葉 (hair blade).

1) 毛球上位 a=b=27.9μ→脂腺位以上 a=b=24.3 μ,

毛小皮の部分は色素なく，核形質1個鮮明であるが，その他の皮質，髄質細胞は色素の為め暗色でその数は不明である．

2) 毛球上位 a=29.7 μ，b=26.1 μ，c=d=6.3 μ→脂腺位以上 a=26.1 μ，b=24.3 μ，c=d=6.3 μ.

毛小皮の核形質4個，皮質，髄質は不明．毛柄及び毛葉のR切片より，毛小皮の核形質は毛球部では短楕円，毛球の上位では長楕円形の豆形で，小型毛ではコンマバチルス型に彎曲する場合あり（写88図C印）．やや上位では扁平に萎縮している．尚これ等の断面によると毛小皮の核形質は皮質，髄質の核形質より早期にヘマトキシリン，エオジンに染着性を失って，角化の早いことを物語っている．

考 察.

前述の観察結果のように，腹部下毛の大部分と背部下毛の一部の捲縮毛の断面では必ず中心となる髄質は中央からやや螺旋の内側に（彎曲の内面）偏っているが，この下毛の彎曲については角化途上において形態的な変化を起す素因があるものとして次のように考えられる．

最小下毛では毛球部の細胞の集りは図（写86図）のように極めて小数であり，毛球の上部断面の観察では数個の細胞の塊にすぎないことが明瞭である．これ等の細胞の角化前の配置を図よのうに仮想すれば（写86図）初め髄質になる細胞を中心に皮質，毛小皮となる数個の細胞が1重に円陣をつくっているとし，その内毛小皮細胞は扁平になって他の細胞を包囲する形態になるので，今まで毛小皮細胞の占めていた位置の一部は隣に接している皮質の細胞形質で埋め合せられるとしても，毛小皮の核形質は初め卵円形で皮質の細胞形質の移動をさえぎり多量の侵入を許さない．核形質の角化は前述の，髄質の核形質と同様にそれは他の細胞形質より遅れる．従って皮質の細胞形質は角化進行により流動性が減小してそのまま経過するため，髄質の中心からこの部分の皮質細胞形質の厚度は常に他に較べて狭くなることになる．

反対に他方の皮質細胞形質の量は常に多量に蓄積されるので（但し厚い部分も髄質を取り巻いている核性質は1重に列んでいる），

遂に毛幹は歪を生じて皮質細胞形質の少量の側に彎曲することになる．

これを側面から観察すると，毛小皮の細胞形質は左右に伸びて皮質を抱擁する態になり，

毛小皮の細胞形質は彎曲外面に少量で図のように傾斜する結果になる（第57図）.

尚お毛小皮細胞の核形質の染着は皮質，髄質細胞の核形質よりも早期に消失するので，毛小皮の細胞形質はやや速かに角化して，彎曲内面を支える支柱の役目を果すものと考えられる.

又毛小皮が縄紋型に極度に傾斜することは，皮質の薄い側に核形質が位置を占めて，上方に向って成長（移動）しており，細胞質形の両端は毛幹を包囲する為めに横に両側に伸長しながら上部に移動するので，核形質を含む部分の成長が速く凸縁部となり，包囲の為めに両側に伸長する部分は常に成長は遅れることになり，凹縁部となるとも考えられる.

又毛の旋回の内側の毛小皮の凸縁部は合せ目がなくて，外側に当る毛小皮の凹縁部に合せ目が常に存在していることもこれでうなづかれる．また最も細い下毛の毛小皮が冠状に毛柄を包囲して冠側に合せ目が少ないことも，包囲速度がはやいために細胞形質が完全に融合して角化する為めであり，逆にやや毛柄の肥大した下毛では合せ目が多くなるとも考えられる．以上により毛の横断面が少数の細胞から成立する小型の下毛の場合は角化期の1個の毛小皮細胞の占める空間の影響は大きいことになる．更に大型下毛において，皮質，髄質細胞が増加し，毛小皮細胞が複数になれば互に相殺してその影響は減少するようである．以上のように考察すると，腹部下毛のように略定形的な捲縮のある最小下毛の毛小皮は，個々に単一細胞から形成されていて，その角化前の狭い毛球の上部においては細胞の移動は略定位置に固定されていることになる．さらにやや大型下毛の場合は，多数の母細胞によるため毛小皮細胞の発生の位置は個々に多少の移動が起るものと考えられる．（最小下毛の場合は次の事実は発見出来ないが），即ち𝕽切片を検鏡の際に同じ視野に毛小皮の核形質は明らかに1個現われているが，焦点を変えると毛小皮の薄い円に沿って略刻面の位置に別に1個の核形質が現われる.

（この𝕽切片の厚度は約20—40$\mu$であるから，毛小皮の幅を略10$\mu$として数個の毛小皮の細胞が上下に重なっていることになる.）

これは毛柄が大きいもの程これを取り巻く毛小皮が複数に変化する徴を表わしているものといえる．従って最早毛柄の捲縮は定形的でなくなり，柄幅が約20$\mu$ではその毛小皮の連絡も縄紋型の冠状ではなく，冠側の低い単純冠状を表わしている．しかるに毛葉は大型下毛，分岐毛の場合でも横断面が円形及び不整円を示す内は，細目の縄紋型冠状の毛小皮細胞の連絡を表わしているので，この毛葉の毛小皮は発生時には，小型下毛の毛柄と同様に毛球の略定位置から分離した単一細胞から成立しているものと考えられる.

以上のように，小型下毛の毛柄は略定形的に捲縮するとしたが，尚単一細胞の冠状毛小皮で取り巻かれる場合で，柄幅がやや増加すると，数個宛交互に反対の位置から連続して発生するか，対面して1個宛交互に，又は多少ずれちがって隣接して発生するかのように，成長と共に毛小皮細胞の発生位置が毛の周縁を移動するので，捲縮は不定形になり，遂に毛質の歪は短い部分で互に均衡を保って，毛柄は直線的になる.

さにら毛柄が肥大した上毛では皮質，髄質は増量して毛小皮は複数になり，同時に異る位置から多数の毛小皮が成長するので瓦列状に発達して毛柄は直楕円筒形に硬直する（写101図）.

尚小型毛の毛柄，毛葉を通じて皮質細胞の髄質包囲が並列一重の包囲陣である内は断面は略円形を保っており，この包囲陣形が乱れ始めると不正円から楕円に移行する徴であり，

ここに下毛から上毛に移る境を思わせるものがある．この時髄質は1個1列型で，毛小皮も単一細胞で毛柄を被ふことが考えられる．

岡島，池田等（1955）はアンゴラ兎毛の捲縮と毛小皮の関係を研究の結果，"繊維の彎曲している部分のスケールの傾斜は外側から内側の根元の方向に向って斜下に走っている．捲縮の原因が彎曲外側におけるスケールの過大生長によるものと考えれば当然繊維はスケールの生長不良なる内側へ彎曲することが考えられる．従って cortex の外側は引伸ばされ，内側よりも配列がよいという事実も説明される"，として筆者の Nutria の場合と反対の現象を述べている．

又堀尾，近藤等（1953）は羊毛繊維の捲縮が繊維の断面の部分的不均質によるものと考えた．

これを更に化学的な裏付けを与えたのが E, MERCER（1953）で彼は捲縮羊毛の外側と内側との反応性の異ることからこの部分に夫々 Ortho cortex 及び Para cortex なる名称を与えた，O-cortex はシスチン約8—9％を含み軟かく，P-cortex はシスチン約15％で硬いので固化の遅れる Ortho cortex が伸びやすく，早く固まる Para cortex が伸び難いので Ortho cortex を彎曲の外側として羊毛の crimp は形成されるとした．

HAUSMANN は前述のように毛小皮の配列について分類しているが，Nutria の場合は下毛では，彼のいう simple coronal scales（単純冠状鱗）と serrate coronal scales（鋸歯冠状鱗）との中間型の冠状毛小皮を示しており，分岐毛以上の小型第2上毛では crenate imbricate scales（鈍鋸歯瓦列状鱗）と flattened imbricate scales（平縁瓦列状鱗）の中間型を示しているが，大型上毛になるにつれて複雑な鋸歯状鱗の複合した瓦列状毛小皮を表わしている．

又 HAUSMANN の scale index（毛小皮係数）はがいして，下毛による異種間の比較を述べているが Nutria の場合では最小下毛から最大上毛まで，その毛小皮を単一細胞とみて，毛小皮の幅は10—20 $\mu$ であり，毛柄の幅は10—200 $\mu$ であるので，その scale index は粗大毛程小さいことは明瞭である．

摘　要

1) 下毛の捲縮は，毛の発育中に毛球の上位において下毛小皮細胞が（特に核形質）皮質細胞の均等配置を妨げることから起る．

2) 背毛，腹毛ともに，定形的な捲縮のある下毛の太さの限界は柄幅で約13 $\mu$ で，単一の毛小皮細胞で毛柄を完全に包囲する冠状毛小皮の限界は柄幅約20 $\mu$ で，毛葉の縄紋型冠状毛小皮の限界は葉幅で約25 $\mu$ のようである．

3) 毛小皮が冠状で単一細胞のときは，髄質細胞も縦に1列配置をする．

4) 毛柄の横断面で，毛小皮，髄質，皮質細胞が単数から複数に（皮質細胞は1重から2重に）移行する境が，下毛と上毛の岐れ目にあたり分岐毛的性格を表わしている．

5. 毛の分類の総括

1) Nutria の毛を次表のように分類する（第30表）．

2) Nutria の背毛は上毛から下毛までの間に，変異に富んでいて，陸棲の毛皮獣に似ており，腹毛は上毛，下毛との間に変異は乏しく，上毛は長大毛を欠いていて，水棲の毛皮獣の特徴を備えている．従って上毛を抜き去り毛皮として利用する際に，背毛叢は不揃いで品質が劣るので，背線を切開して腹毛を中央に置いて代表させることになる．

第 30 表

| 分類 | | | 毛長 cm | 柄幅 μ | 葉幅 μ | 毛量 % |
|---|---|---|---|---|---|---|
| 背毛 | 第1上毛 | | 2.0～8.0 | 15～125 | 20～200 | 5～10 |
| | 第2上毛 | | | | | |
| | 分岐毛 | 分岐上毛 | | | | |
| | 下毛 | 分岐下毛 | 2.0～2.5 | 12～14 | 12～25 | 95～90 |
| 腹毛 | 第2上毛 | | 1.5～5.0 | 15～85 | 20～120 | 1～5 |
| | 分岐毛 | 分岐上毛 | | | | |
| | 下毛 | 分岐下毛 | 1.5～2.0 | 10～12 | 10～20 | 99～95 |

## VI 発生と換毛

### 1. 毛束と毛群及びその名称について

毛群. MEIJERE (1894) は哺乳類の被毛は中央に大型の上毛を配した3原毛 (original trio group) を基形に変形としてそれ以上の数個の原毛を以って1毛群とするもの，又その原毛を中心に分芽，分根によって毛束を形成しているものなどを述べて，毛群 (hair group, Haargruppe) について基礎を確立している．その後多くの哺乳類の被毛に関する研究は Meijere の説にならっている．

SPENCER や SWEET (1899) は単孔類 (ハリモグラ，カモノハシ) ではどの毛群も中央毛から分芽 (budding) によって発生するとしている．

NOBACK[30] (1951) は有袋類，食肉類等の毛包は凡て独立に胚芽層 (Stratum germinativum) から皮内に陥入するという．WILCOX[43] (1950) は chinchilla の成獣で中央毛の両側の2個の毛束は1個の毛包から分芽的に発生しているとした．阿部[2] (1920) は牛の被毛の研究で，牛の毛包の発生の模写を記載し又エチゴウサギの胎児の被毛について，発生中の毛原基の写真をかかげて副毛の分芽が原毛の毛包の上位で起る場合を述べているが，3原毛の発生については全くふれていない．

又羽部，上坂等[17] (1943) は兎毛の胎児の毛原基の発生過程について詳述しているが，胎児期の毛群の構成については記載がない．

GALPN[15] (1935) は緬羊 New Zealand Romney の胎児で研究の結果，肩部から背部では56日で中央毛を，76日目には両側毛が増して3原毛群となり，97日—111日目には副側毛を追加して9毛包1毛群となり (nine group)，それ以後は幼毛は表皮外に現われて生毛の態勢を整えて出産すると報じている．凡ての毛群は胎児の体表の重な個所では (9毛包1毛群) 毛であるとしている．

毛束. MEIJERE[31] は1毛包から生じて同一毛孔内に一束となる場合を真毛束 (echte Bündel) と呼び，この場合は皮脂腺の開口部下方はやや長い共通の毛包であること，次に相隣接した毛包が皮脂腺の開口部の上方で同一毛孔 (hair pore) で毛束を成す場合を偽毛束 (falsche Bündel) と呼んでいる．

阿部[3] は牛毛の発生の研究で，原毛より副毛が分芽する場合は原毛は表皮面に近い部分で分芽して副毛を生ずるためその共有する毛孔の部分は極めて短いので隣接していた別個の毛

包から生じて同一毛孔で毛束となったものと区別し難い，これも又 MEIJERE の所謂る偽毛束と呼ぶか否なか解釈に苦しむとしている．

DRY[41] (1926) は mouse の体の主要部分では1毛包に1毛以上の発育毛を観察したことはないという．但しこの毛管内に成毛が毛棍（棍球）(hairclub) を結束して一束を形成する場合があるが，この時はこの毛棍は毛世代の順序に $G_1$, $G_2$, $G_3$, と低位置にあるのでその毛世代が明らかに判定がつくといい，1毛包から2－4毛生じて毛束が出来ているが，棍状毛を3個以上含む毛束の場合は発育毛を持っていないという．これを DRY は毛束 (hair bundle) と呼んでいるが，この毛束について WOIBACH[42] (1951) は複合毛包と呼ぶべきだとしている (Multiple haired follicle should, in my opinion, be called "compound follicles")

毛群の形成の観察

Nutria の3ケ月胎児の背皮切片資料で調査の結果明らかに生毛群は3毛包群→7毛包群→15毛包群の発生が立証される．

出生直後（2日目）では生毛群は数量的に確認され，3毛包群37.5%，7毛包群16.7%，15毛包群1.4%で平均5.6毛包群である．次は1毛群の毛包数序列

37.5% 16.7% 1.4%
③ →4→5→6→ ⑦ →8→9→10→11→12→13→14→⑮

15生毛群，即ち最多毛包群の形成を例に取れば次のように3ケ月胎児期より生後1ケ月頃まで生毛群が明瞭に認められる．(写7—20図)

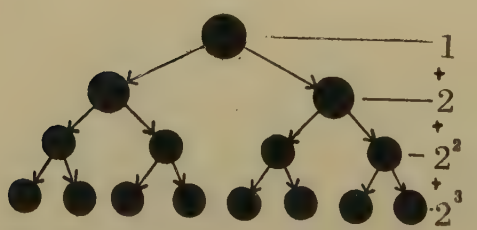
中央毛包を中心に側毛包2個を生じ3原毛群 (original trio group) となり，この各側毛包の両後側に副側毛包を生じて7生毛包群 (7 follicles group) となり，更にこれ等の副側毛の両後側に副側毛包を生じて15生毛包群 (15 follicles group) となる．R切片資料による調査の結果と毛の分類とを対照して，背部生毛群は次のように分類される．

(1) 3ケ月胎児より生後1ケ月までの毛群．

1) 15生毛群，最多毛包の毛群

$1+2+2^2+2^3=15$

第 26 図

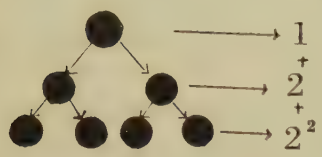



TOLDT の狐の被毛の分類と対照すれば次のようである．

中 央 毛, Leithaar, Leit-Grannenhaar,
側 毛, Dicke Grannenhaar, dünne Grannenhaar,
第1副側毛, Grannen-Wollhaar,
第2副側毛, Wollhaar,

2)　7生毛群　$1+2+2^2=7$

第　27　図




3)　3生毛群　$1+2=3$

第　28　図

生後1ケ月では毛束の形成は稀れで2次副側毛と生毛との2個が複生の場合があるのみで大多数は成毛又は成毛と青毛との混生の毛叢が完成している．又L切片によれば後継毛の毛芽の発生開始も明らかである．

(2)　生後2ケ月，3ケ月，4ケ月，8ケ月，12ケ月と12ケ月以上の毛群，

R切片による毛束の解体について調査の結果生後2ケ月→8ケ月まで2次副側毛包（毛母基）の新生があるが12ケ月以上では毛包の増加はない．

第31表　生後2ケ月より8ケ月までの毛包数の変遷

| 背　毛　包 | 2ケ月♂ | 3ケ月♂ | 4ケ月♂ | 8ケ月♂ |
|---|---|---|---|---|
| 1毛束の毛量最多数 | 7 | 7 | 10 | 9 |
| 最高頻度毛量 | 3 | 5 | 7 | 5 |
| 平均毛数 | 3.5 | 4.3 | 5.6 | 5.1 |
| 毛包数 | 2.3 | 2.7 | 3.5 | 3.1 |

第32表　成獣の場合，月別における毛包数の変遷

| 背　毛　包 | 1月 | 2月 | 3月 | 4月 | 5月 | 6月 | 7月 | 10月 | 11月 | 12月 | 平均 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1毛束の毛量最多数 | 15 | 11 | 14 | 14 | 15 | 13 | 15 | 14 | 16 | 17 | |
| 最高頻度毛量 | 8–9 | 7–8 | 8 | 7 | 10 | 8 | 9 | 9 | 9 | 12 | |
| 平均毛数 | 8.7 | 7.1 | 8.7 | 7.8 | 8.8 | 7.6 | 8.4 | 8.8 | 8.9 | 9.5 | |
| 毛包数 | 4.2 | 3.9 | 4.0 | 4.4 | 4.7 | 5.8 | 5.4 | 5.9 | 4.9 | 5.2 | 4.8 |

以上のようにNutriaでは生後2ケ月より8ケ月までは毛束内の毛包の増加がありその数は2強より3強である，12ケ月以上では毛包の増加はなくその数は4より6弱で平均4.8≒5である．尚別途調査の結果，12ケ月以上の成獣の1毛群の毛束数の平均は9.75≒10で中央毛の単生毛包を含んでいる．よってこれ等毛束の調査による1毛群の平均毛包数は

$$\underline{\text{側毛，副側毛}}\quad\underline{\text{中央毛}}$$
$$5\times 9\ +\ 1\ =46$$

で，直接毛群の毛数調査による場合は平均毛包は45で，ほぼ一致しているので，Nutriaの成獣の背毛包は1毛群平均45→46とする．

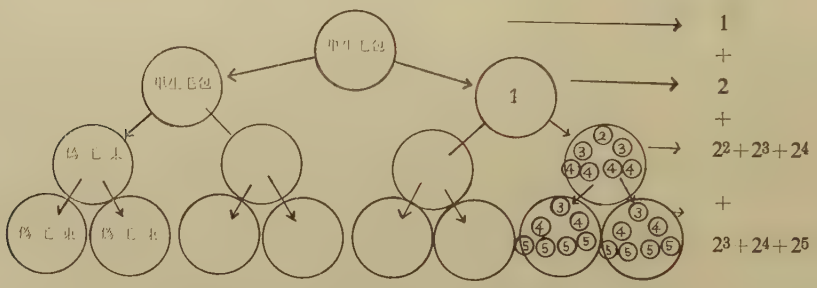
R切片で観察の結果背部1毛群の毛束数（中央毛，側毛包を含む）は3毛束より17毛束ま

であり，概して生毛群の毛包数に一致している．

生毛（ウブケ）の最多毛包数15毛群の場合，2次副側毛包は第1副側毛包及び第2副側毛包の生毛を先頭に順次隣接して発生して偽毛束を形成するが，中央毛，両側毛の所謂る3原毛は多くの場合2次副側毛を伴う偽毛束を形成しない．但し（1－2毛包）の小型毛束が毛群の前方附近に（動物の頭部に向って）屢々散在している（写117図）

この為め15生毛群型の15毛束毛群となるべき毛群が17毛束毛群とも算定されるのである．図（第29図）のように二次副側毛の最多毛の毛束も生毛の発生の方法に準じて2のn乗で，その毛包数が増加すると推定される，又7毛束毛群の場合は中央毛は多くは大型毛群の側毛に準じて2次副側毛の発生を観ない．又3毛束毛群の場合は一般に3毛束とも偽毛束の形をとる．

第　29　図



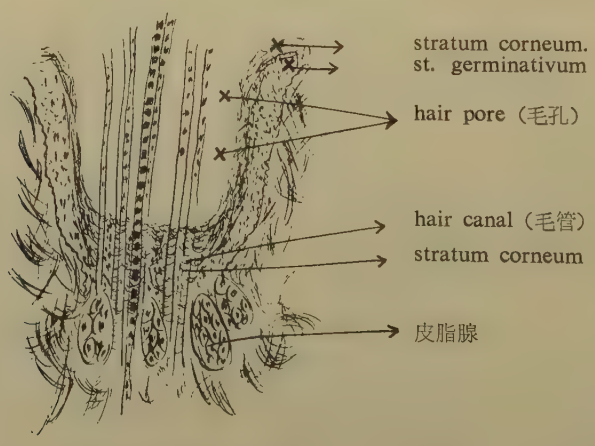

1毛群，15毛束の最多毛包＝$1+2+2^2+2\times(2^3+2^4)+2^5=87$

1毛群，7毛束　〃　〃　＝$1+2+2\times(2^2+2^3)+2^4=43$

1毛群，3毛束　〃　〃　＝$1+2\times(2+2^2)+2^3=21$

毛束の形成観察．

第　30　図




Nutria では生毛（lanugo hair）を先頭にその毛包に隣接して新毛原基（毛芽）が陥入して相接して発生した副側毛は個々に角原層（stratum corneum）の毛管にまかれて単生又は

棍状毛とその後継毛とが対生或は棍状毛2，幼毛1の3毛世代が連生して所謂真毛束を形成する．

これ等の真毛束の集りを取り巻く結合組織が隆起し窪み（凹処）を作って広口の毛孔（hair pore）を形成する．これが所謂偽毛束であろう（写 116, 123, 124図）．Nutria の場合は Dry（1926）が mouse で観察したように，L 切片では毛束の観察は困難であるが，これをR切片で試みる際2個が棍状毛根で1個が青毛か又は幼毛の3毛世代連生の場合は最古参の毛世代の毛根は毛棍の個所を切断されているので，萎縮した刷子様の毛棍の断面は星芒状であり，次代の棍状毛は無色の毛根部を切断されているので円形を保っている．これ等3毛世代は明らかに順次に判別しうる（写121, 142図）．

尚季節的変化について述べたように1毛包による世代毛束（generation hairs bundle 筆者仮称）は3連生毛束が最高で又この3個の中2個は棍状毛で残る1個は青毛乃至幼毛の発育毛である．即ち3個が凡て成毛の連生毛束（T. $GnO, +Gn+1O, +Gn+2O.$）はない．この際概して集団の先頭にあって外郭に接して位置をしている生毛包がやや大型な上毛の場合は，皮脂腺或はその毛包の占める容積が過大なためにその結束が緩み遂に偽毛束から離れて独立の毛包となることが多い．

又相接した毛包は個々に皮脂腺を保有しているようでこれ等の皮脂腺は毛包集合の間隙に容れられず外側にはみ出して毛束の集団を包囲している（写123, 124, 126, 132図）．

## 考　　察

筆者は Nutria の3ケ月胎児の背部L切片で写真（写15図）のように原毛の発生中の毛原基をとらえたが，これは正側面からの写真であるので，3原毛包が重なり合っていることが考えられる．又別に毛群を背線を横断して毛包に平行（斜行）なS切片で観察すると写真のように体表に現われたばかりの3原毛幼毛の先端（芒先に当る）又はまた現われんとしている巻き込んだ鞘毛の先端を観察した（写8, 9, 10, 16, 19図）．

これ等は各個に角質膜を内壁にした毛管（hair canal）内に独立している．又この3原毛中心に（R切片で観察，7—15毛包）数個の毛芽は平行して表皮面から陥入しているので，中央の毛包を主幹にした樹木のようである（写7図）．

又 Hämatoxylin の染色無処理の表皮の透視写真でも3原毛群は黒褐色の鞘毛の尖端は毛管から体表に現われる際の角質層破壊の有様を示している（写9, 19図）．

以上よりこれら中央毛，両側毛の3原毛が同じ毛包から所謂分芽することは考えられない．更に側毛を挟んで発生している樹枝状に染っている副側毛の毛原基も同様に考えられる．即ちこれらの毛包は中央毛，側毛，副側毛の順序に隣接して胚芽層から毛原基が陥入して毛群の基礎が確立するようである．Nutria の場合は既に3ケ月胎児において生毛の毛包数は決定的であって，単独の生毛で構成された毛群は生後約1ケ月までほぼその形を保っていて，毛束はその後の下毛的副側毛の発生によって成立するのである．

Butcher[8]（1950—51）は上皮性の毛芽の多くは胎児時代か生後間もなく胚芽層から形成されるので毛原基が生後遅くまで発生することは疑問であるとした．筆者の Nutria の場合は生後約1ケ月までは体表にある被毛は生毛（ウブゲ）の成毛と青毛で構成されており，体表からの観察ではいまだこれを中心に発達すると見られる毛束は稀れである．ただし毛皮の切片の資料より観察せば成毛の毛包は既に毛芽として後継毛の発芽もあり，又2次副側毛として下毛

な新生毛原基の増加が認められる．これが生毛を中心に毛束を営む兆は明らかである．

更に生後2, 3, 4, …8ケ月と生後長期に亘り2次副側毛包（postnatal sublateral hair follicle）の増加が認められるが，満1ケ年後には新毛包の増加はない．毛群の毛包は成獣に成長するまでに6倍強になるが，生後2ケ月間の増加が最も多く生毛の2倍弱で後は10ケ月間程で補充されている．

又上坂[41]等（1950）は兎毛皮の連続切片によって研究の結果，遂次新毛包は原毛包として次の新毛を分芽する毛束の形成過程を詳述している．筆者の Nutria の場合も同様に胎児期に発生した生毛群の毛包に続いて2次副側毛包の発生がありこれ等が毛束を構成するのであるが，新毛を原毛としてその毛包から次の新毛が分芽する事実は見られない．生毛の発生と同様に新毛の毛包に隣接した胚芽層の陥入により次の新毛包が発生するものと考えられる．

2. 換毛の生態

筆者の調査資料では Nutria の毛世代の週期において Dry[11](1926) や Chase[10](1953) の所謂発成期（anagen），衰退期（catagen），休止期（telogen）を終始追求して観察することは出来なかったので，その各期の一齣を捕えて断片的に検討を加えた．Wolbach[42] (1951) は mouse の被毛の休止期の終りに，毛包が萎縮を起す直前に，毛母基は乳頭を取り巻く毛球の部分で最後の増殖が行われる，これが毛棍と乳頭との中間にある上皮索（epithelial cord, hair stem, Epithelstrang）に存在して次の毛世代の毛母基を継承することになる．又その上皮索の先端に萎縮消失からまぬがれた乳頭があるこれを休止乳頭（resting papilla）と呼び毛母基とともに次の活躍を待機している．検鏡観察によれば Nutria では衰退期の初期の毛球は写真のように乳頭上方の広範囲に亘って細胞核が卵形で Hämatoxylin に濃染していて，未角化状態を示し，その上方に瓜実型に萎縮し半ば角化の核を混えている（写64, 65図）．ここで発育毛の毛球を比較対照の例に採れば写真（写104図）のように紡錘形の毛乳頭を包んで囲繞している毛母基から分裂した細胞は乳頭の頂点を離れると直ちに角化開始の状態にあり，凡ての核は卵形より細長い瓜実型に変化している．上記衰退期の毛球では球形に萎縮変形開始の乳頭を下方に残して角化しない毛母基は上方に移動して柱状（column of cells of matrix origin）を示している．

以上により最後に増殖した細胞は角化を停止して毛母基として残留することが明らかである．

更に Wolbach[42] (1951) によれば mouse ではこの上皮索は硝子様膜（Stöhr は結合組織から，Wolbach は上皮性のもの）に包被されてこの膜の肥厚と収縮と，上皮性細胞の萎縮とともに上皮索は褶曲を作って毛乳頭は毛棍の下底に接近して休止すると言う．Nutria では衰退期の終期に Dry[11] の所謂毛の発生機能を包含する萎縮した小嚢（saccule）は短縮した上皮索の先端で写真のように鍵型に彎曲して小嚢の下端は垂れ下り，その鈍端に萎縮した乳頭がある（写69図）．

残留色素（residual pigment）は常に Dry[11] の言うまばらにこの鍵型の彎曲部に存在し，これとともに毛母基が休止している．

発成期（anagen）の乳頭の定形は紡錘形であるが衰退期，休止期では引き締った球形で，Hämatoxylin の染りは発成期に較べてよくない．次代の後継毛の毛芽の発成期は休止毛母基の分裂再開より始まり（写67, 70, 72図），色素細胞の増殖を伴なって萎縮乳頭の上部を半円形に色彩るので毛芽の発生開始は明らかである（写73, 74図）．これと同時に萎縮して

いた乳頭の細胞塊が紡錘形の定形に復して毛原基の発生時と同様に活潑な毛母基を頂き上皮索の分裂再成がしきりに興り結合組織索（Haar stengel）に沿って斜入し，青毛期毛包の位置に落ち着くのである．SEGALL[11] 等はこの Haar stengel を真皮性の組織から成るとしているが，DRY[11] は mouse の毛包には一定の真皮性の毛根鞘があることを疑問として Haarstengel は外根鞘から成っていて上皮性の組織であるとした．筆者は球毛がこれに沿って下向するのを写真で捕えることが出来た．以上より Nutria の場合乳頭は破壊されず休止期から覚醒して定形に戻ることは，WOLBACH[42]（1951）の mouse の休止乳頭（resting papilla）と，DRY[11]（1926）の mouse の新乳頭形成の意味と一致する．

又これと同時に上皮索の上部旧毛包下端の曲り角附近の母細胞（人毛では STÖHR はこれを Haarbeet 毛榻と呼んでいる）は棍状毛の斜面上に接触して上方に向ってこれに平行して増殖し皮脂腺位の毛管の下底で止る（写75，78図）．毛の尖端はこの附属新毛包内を進み皮脂腺と棍状毛の毛包との接着部である毛管の下底を通って毛管内に入り棍状毛と合流して対生する．この際後継毛は棍状毛を下方より押上げることは全くない（写78図）．又上毛では後継の新毛は常に先代成毛の傾斜面上に背負われたように接触して成毛の前方（頭部に向って）にある（写75，78，93，97図）．

毛管内で新毛が反転して側面に出る場合もある．尚 CHASE（1954）によれば mouse の毛包の傾斜角は皮面に対して 30° とあるが，Nutria の場合は背腹ともに略々 20°→30° の間にある（但しホルマリン漬生皮の場合である）．Nutria では上述のように後継毛は先代毛を庇護するように発生している．

上毛の場合後継毛ははじめ芒先は細く次第に大きく幅を増して毛葉部では最も拡大して緊張するので先代毛根と緊密に毛管内に詰め合っているが，成長が進んで毛頸部になると急に後継毛の径幅が縮小すると同時に毛管が異常に緩んでくるので先代棍状毛の落毛はこの時起る．これは独立毛包で中型上毛，大型上毛の場合である．

又寒冷季，温暖季の別なくこれはあてはまる．小型上毛は毛柄（hair stalk）まで対生する場合が多いが（P. GnO, Gn+1 Y.），両毛共に棍状毛（P. GnO, Gn+1O）の場合は少ない．極めて稀れであるが棍状毛 2，幼毛 1 の 3 連生毛の特別な例もある（T. GnO, Gn+1O, Gn+2C）．下毛の残留生態については“換毛期の発見”で述べる．

註．DRY[11]（1926）は mouse の被毛で次のように hair stalk を述べている．筆者は Nutria の被毛でこの hair stalk を毛柄と仮称して引用する．DRY の ‘The coat of the mouse’ の 291 頁に次のようにある．

Auchens or Type C. This type differs from the last in having a septate constriction of oval cross-section at about two-fifths of the length of the hair from the apical end. This lies between the distal “blade” and the proximal “stalk”.

又石沢[24] は組織学提要，第 2 巻，148頁に Haarstengel に hair stalk を当てているが筆者はこれを用いない．

又 WOLBACH の ‘The hair cycle of the mouse and its importance in the study of sequences of experimental carcinogenesis’ の 521 頁に次のように述べて，The product of this last proliferative activity constributes to a column of cells of matrix origin, the hair stem (“Haarstengel” of German authors), which later lies between the hair club and surviving cells of the papilla. 上皮索（Epithelstrang）を結合組織索（筆者仮称．Haarstengel）と混同している．

### 3. 換毛期の発見

一般に春秋 2 季に換毛を繰り返えす動物の被毛は夏毛，冬毛と自から性格が異なり，両者

の区別が明らかである．従って動物の発育が常態である限り，盛夏厳冬においてはその季で特徴のある被毛を完全に装った後は，交代の新毛の発現は見受けられない．その最も明確な事実は阿部[4] (1917) のエチゴウサギの夏毛と冬毛の場合である．よって盛夏，厳冬の2つの資料について調査，観察せばその動物の換毛の推移の傾向が略々判断されうるとみて，7月20日と12月24日に屠殺処分して獲た資料についてℝ連続切片を作って観察して次のような結果をえた．

(1) 夏季牡成獣皮のℝ連続切片による観察成績

動物記号 N—7. (1954, 7月20日屠殺処分♂ 5 延)

切片順序 No. 1, 最上層の表皮面で毛孔の開口部を表わしている．毛群の基礎とみられる3原毛群 (original trio group) の中央毛と両側毛の上毛管3，その他独立の小上毛管2，下毛を包む偽毛束の毛孔9で，大小上毛5，下毛66計71から成立していて，長幅 0.8 mm×短幅 0.28 mm. の内に14毛束 (14生毛型) の毛群を形成している．その内訳は中央毛にあたる，中空で皮質の内縁とそれに附着する髄質の小片が濃褐色の色素顆粒に富んでいる第1上毛 $\frac{b}{a}=\frac{65.7\mu}{155.7\mu}$ の暗葉毛 (毛葉腹と推定) が毛群の中央に位置し，左側には原則的に3原毛群の側毛である中空，内縁濃褐色の小上毛 $\frac{b}{a}=\frac{31.5\mu}{60.3\mu}$ の毛頸，中心に小量の褐色素顆粒を含む角化した棍状毛根 (成毛根) $\frac{b}{a}=\frac{20.7\mu}{33.3\mu}$，濃褐色の髄部のある小上毛の青毛の毛柄 $\frac{b}{a}=\frac{24.3\mu}{27.9\mu}$ (推定) 等と，濃褐色の髄質のある青毛と黒褐色の幼毛との発育毛 19，中心に色素顆粒少量を含む有色成毛根に，無色成毛根15で，成毛27計46の下毛が分れて6の毛束を形成している．

右側は3原毛群の側毛である中心に少量の褐色素顆粒のある成毛根 $\frac{b}{a}=\frac{36.9\mu}{27.9\mu}$ 小上毛が単生し，青毛8の発育毛と有色，無色の成毛根12計20の下毛が2つの毛束に分れている．

以上のように毛群は中央毛の左右に側毛と副側毛の毛束とが加わって鳥が両翼を張ったような形である．

No. 2, この場合毛群の深度は 0.2mm で，長幅 0.8mm×短幅 0.30mm 内にある．3原毛群に明らかな変化はない，左側に脂腺が現われ，偽毛束の解体が始まる．右側は対生毛1対，左側に8対が分離している．有色成毛根は減じて12，無色成毛根は増して27計39で，数の移動はなく，発育毛には変化はない．

No. 3, 深度は 0.2 mm→0.4 mm の間にあり，長幅 0.76mm×短幅 0.32mm 内にある．3原毛群に明らかな変化はない．両側に皮脂腺あり，下毛の対生毛は右側に7対，左側に16対となる．下毛の有色成毛根は5に減じ無色成毛根は34に増して，計39で数の移動はない．幼毛が幅を増している外に発育毛の変化はない．

No. 4, 深度は 0.4 mm で，長幅 0.76 mm×短幅 0.28 mm 内にある．3原毛群及びその他の上毛に変化は少ない．両側に皮脂腺あり．対生毛 7, 16, 計23対である (No. 3 と同様)．有色毛根 4, 無色毛根 35, 計39で，数の移動はない．発育毛に変化はない．

No. 5, 深度 0.4→0.7 mm の間にある．長幅 0.76 mm×短幅 0.32 mm 内にある．中央毛は僅かに両径を減じている．右側毛1と左副側上毛1の成毛根2は棍球に変っている．皮脂腺は全く消失し，対生毛は解体している．下毛の有色毛根は凡て周縁は緩かな波状のしわを生じて萎縮している．幼毛，青毛ともに径幅を増している．

No. 6, 深度は 0.7 mm で，長幅 0.68 mm×短幅 0.28 mm 内にある．3 原毛群などの発育中の変化は少いが，小上毛の成毛根はさらに萎縮して周縁は波状の無定形である．下毛の有色成毛根1，無色成毛根16，計17で，成毛根 22 を消失したことになる．無色成毛根はさらに萎縮して星芒状である．球毛の芒先が 1 つ現われて，その他幼毛，青毛の発育毛は凡て径幅を増している．

No. 8, 深度は 1.0 mm で，長幅 0.6 mm×短幅 0.28 mm 内にあり．左側は 2，右側は 1 の毛包の集団になる．3 原毛群の発育毛は長径が短くなって円形に接近している．側毛の萎縮成毛根 1 は消失し，1 は無定形の痕跡を留めている．

下毛の成毛は全く消失して，幼毛3，球毛の毛球とみられるもの2，その他青毛は径幅を増して毛球に接近しているが数の移動はない．

No. 9, 深度は 1.3 mm で．長幅 0.52 mm×短幅 0.32 mm 内にあり 3 原毛群の発育毛は長径が短縮して円形に近い楕円形になり成毛根は消失して全くない．毛芽，球毛は消失し幼毛，青毛計27の発育毛は径幅を増して濃色になり毛球の上部とみられる．

以上を総括すれば表皮面では下毛の発育毛27，成毛39である．深度 0.2—0.4 mm では発育毛23，成毛23が対生毛となっている．深度 0.7 mm. では鞘毛の芒先が成毛と対生しており，0.7—1.0mm では毛球 2 つが現われたので発育毛30となる．球毛の毛球と観られるものは成毛根が消失する附近の深度で発見されている．Ⓡ切片が密に連続すればその数を増したかもしれない．表面から深部に達している27の発育毛の内23は成毛と対生している．単生する 4 は既に成毛が脱け落ちたものと推定する．（季節的変化で詳述するが生後満 1 ケ年を経過すると幼毛は凡て対生毛であり，青毛のみに単生毛が存在することから，単生毛の青毛は対生する成毛が脱け落ちた後と認める．このⓇ切片は 7 月20日の暑熱季であるが，さらに寒冷季では青毛も凡て対生毛，連生毛であって単生する場合は極めて稀れである）．成毛根39の内青毛，幼毛等の発育毛と対生するものは23で残る16は未だ後継毛を持たないことになる．一方下毛の成毛根の内で表皮面の切片では有色成毛根が18であるがこの内多くは毛根の上部を現わしているので毛球は深部にあってまだ後継毛のない成毛を代表している．しかし事実は皮脂腺下位の切片に球毛 3 が現われたので後継毛のない成毛は13と推定される．なお表皮面に芒先が現われている下毛の幼毛は皮内深部まで黒褐の濃色で，深度 1.0 mm では髄質はあるが皮質に色素顆粒が多く毛葉の腹部を示している．従って幼毛の毛球の毛母基から毛葉の中腹部が形成される頃，毛球はすでに下降を停止しているようである．それは髄質の色素と気室が黒白の縞模様によって毛柄部（hair stalk）を示ている青毛と，黒褐の濃色の毛葉（hair blade）を発生中の幼毛とが入り混じって毛球の下底を揃えて深部に斜行している毛束の L 切片の側面写真と対照すれば明らかに判る．

因みに下毛の毛葉は約 5 — 7 mm. で（毛の分類にあり），下毛の毛球下底は約 1.8 mm で，中央毛の毛球降下深度は約 2 mm で，皮厚は約 2.2 mm である（L 切片による）．

全毛包は皮脂腺位の切片で毛束解体から副側毛の毛包数が43と判り，それに 3 原毛が 3，副側毛の独立上毛が 2 加わって全毛包数48となる．毛包数を 100 ％として全毛数を71とすれば，全毛包数に対して $\frac{71}{48}\times 100=148\%$ となる．

(2) 冬季牝成獣皮のⓇ連続切片による観察成績

動物記号 N—12 ♀ 4.5瓩，1954，12月24日屠殺処分．

切片順序 No. 1 a, の表皮面では中央上毛, 左側 2, 右側 3, の5偽毛束を伴った生毛の6個型の毛群である. これは側毛として独立上毛なく, 分岐下毛らしき大型下毛1或は2を毛束内に包含していて明葉帯 (agout band) を持った分岐上毛はない. 中央毛は根幅 $\frac{b}{a}=\frac{35.1\mu}{38.7\mu}$ で乳濁色の断面の中央に黒褐色素顆粒が散在し, 第2上毛の棍状毛と推定する. 成毛44, 発育毛21, 計65から成る毛群である.

No. 1 の b, c では5偽毛束とも解体を始め, 先頭に大型下毛 (分岐下毛を含む, 生毛包) が遊離し, 順次小型下毛 (2次副側毛) が遊離する徴を示している.

No. 2, 深度 0.15 -0.4 mm は皮脂腺位で偽毛束は解体して成毛と発育毛の対生毛包19 (P. O. Y. 又は P. O. C.), 成毛2と発育毛1の3連生毛包4 (T. O. O. Y 又は T. O. O. C.), 成毛の単生毛包1 (S. O.) となり, 下毛では発育毛で単生するものなく (S. Y. 又は S. C.), 成毛の単生毛は唯1である. 中央毛は成毛の単生毛 (S. O.) である. 成毛45, 発育毛23, 計68である. 皮面より成毛 1, 発育毛 2, 計3の増加であるが, この成毛1は No. 1 では右側の毛束中の成毛がR切片作製時に脱落して減数したものであろう. 又発育毛2は鞘毛の芒先で皮面には未だ現われていないものである. 中央毛の棍状毛根は変化なし.

No. 3, 深度 0.4 -0.7 mm では皮脂腺は消失してその下位に当り左側の棍状毛は凡て消失し発育毛10残存し, 右側の棍状毛は1残存, 球毛の芒光3出現, 中央毛は断面が星芒状の棍球位に当り $\frac{b}{a}=\frac{35.1\mu}{35.1\mu}$ である. 棍状毛2, 発育毛25, 計27である.

No. 4, 右側は不定形の鞘毛球の切片現われ, これを加えて発育毛16, 棍状毛1, 計下毛17, 中央毛は棍球の下底で無定形である. 左側には鞘毛の芒先現われ, これを加えて発育毛11となり, 毛群の全被毛は棍状毛1, 発育毛27, 計28である.

No. 5, 左側に無色の棍状毛根1現わる, これは No. 4 までは青毛として発育毛に扱われていたもので発育停止直後のものであろう. 発育毛9を加えて下毛は10である. 右側は発育毛11となり鞘毛は毛球が現われるもあり, 既に消失しているもあり, 毛球2を合せて13である. 中央毛の棍状毛は消失して下方に潜んでいた発育中の鞘毛が現われている. 毛群の全被毛は鞘毛球を加えて発育毛24である.

No. 6, 深度 1.5mm 最早毛包の下底近くで両側の毛団の区別は出来ない. 発育毛の毛球の上位19, 棍状毛根1, 計20である.

No. 7, 発育毛の毛球位16,

No. 8, 毛球2, 発育毛即ち青毛の毛球で充分に伸びきった毛包で最早下方に移動しない.

以上を総括すれば暑熱季の例と異るところは皮脂腺位においてP. O. O. (又はP. GnO, $Gn_{+1}$O.) 即ち成. 成対生毛包のあること, T. GnO. $Gn_{+1}$O. $Gn_{+2}$Y, T. GnO. $Gn_{+1}$O. $Gn_{+2}$C即ち成. 成. 発3連生毛包とがあることである. 更にこの場合の中央毛は棍状毛として落毛寸前のもので既に下方に後継毛として球毛が潜在して発育中である. その他は夏季の例と異ならない.

(3) 考 察

1) 無色の小型毛根は髄部を欠き角化して水膠色である.

2) 下底から上層の皮脂腺附近になると無色の小型毛根の数が増して来る.

3) また皮脂腺位で無色の棍状毛根の内でも表皮面の毛孔の開口部では, 少量の色素顆粒を含んでいるのが, 有色毛根の仲間入りをするので減数することになる,

4) 皮脂腺附近で棍状毛の下部から発育中の球毛が成長して後継毛として同じ毛管内に

対生する．下毛の有色毛が個々の毛管に単生する場合は対生していた棍状毛がすでに脱け落ちた後の発育旺んな青毛か又は発育中の青毛の末期で発育停止前のものかであろう．

幼毛で単生する場合は成獣では認められない．無色のもの或は中心に僅かに色素顆粒を留める毛根で単生するものは皮脂腺下方の毛母基では後継毛が発生中のものかまたは棍状毛に変形中，即ち成長停止期に入っているものであろう．

5）棍状毛となり下底の毛包組織の萎縮に伴って毛乳頭は萎縮し，皮脂腺下方に集結した毛母基は増殖を始めて発芽の態勢を整えることになり（換毛の生態で詳述する），従って皮脂腺下方に毛芽が現われ，鞘毛（Scheide-haar）は幼毛となって成長しその毛球は下降して一定の深度（1.5 mm）に達して留まり青毛期（anagen の後期）を経過して成毛期（catagen-telogen）に入る，被毛の生涯の生態が明らかに認められる．

6）表皮面では一毛孔に毛束を結成する下毛も皮脂腺位で解体すると単生毛，対生毛等個々の毛包，毛管，内におさまり，互いに接着していて皮脂腺は毛束を単位に取り囲んでいる．その皮脂腺の数は毛束の毛包数に略等しい．完全に周囲を取り巻いているようであるが，そのS切片によると毛束内の毛包の結束が固いので各毛包の皮脂腺は外方にはみ出してその外郭を囲んでいるように観察される．

このように1つの小上毛の副側毛を中心に2次副側毛で形成される毛束もあるが，この毛群の多くは中心となるべき小上毛は毛束に近接しているが皮脂腺が過大なために同じ毛孔に包含されないで前方に独立している．さらに一般に中央毛，側毛などの大型毛はその特殊の場合であると考えるべきであろう．

それは中央毛を先頭にその前に発生した毛包を中心に両後方側に2つづつ側毛から副側毛と後退しながら順序に発生する生毛の毛群単位を考えると，上毛は前方に取り残されて独立する傾向を示している．さらに2次副側毛の発生もこれに準じているようである．

皮脂腺下方に移行するに従って，毛束単位に集結して毛束と毛束との間にあったやや幅広の結合組織帯は細くなり，多くは両側毛包を中心に下毛包，上毛包は密に接近しその間に僅かに結合組織繊維のつながりが観察される程度である．さらに深部になると下毛の毛芽の毛球は消失し，最下底では下毛毛球も消失して，中央毛，両側毛の青毛毛球は胎児期の3原毛群と同じように相接して連立している．S切片と対照すると毛群は下底より3原毛群の3毛包を中心にV字形に上方に向って末広がりになっている．（写116図）．

7）なお下毛の棍状毛が久しく毛群中に留まっている事実（又毛皮として脱毛しない事実はとくに鞣製によって毛包はよく締ってくる）は下底より幼毛が速かに追随してきて皮脂腺下方で追い付き（写79. 80図），角化した棍状毛下底の被膜をさけて鞘毛の芒先は棍状毛に平行して進みついに棍状毛根の留まる毛管の下底より内部に貫通して狭い毛管内に対生すると共にさらに隣接する数個から10数個の毛包が集って1つの毛孔に充満し1條毛束を形成して，互いに締めつけられて2段構えの結束をするので，成毛の脱落を遅らせる結果となる．なお棍状毛の棍球は僅かに先き太となり表面に粗面を生じて被膜内に固着している（写77図）．

さらに寒冷季では成毛は1毛包内に2世代と発育毛の1世代と加えて，3世代に亘って残留する場合が多くあり保温に役立っている．大型上毛は寒暖の別なく後継毛の毛頸部が成育する時期まで（幼毛期）成毛と対生する場合は稀れである．小上毛は下毛に準じている．大型上毛については“換毛の生態”で述べてある．

第33表 連続切片による背部毛群の観察

動物番号 N—7

| 切片の順序 | 皮面からの深度と位置 mm | 毛群の大きさ 短径/長径 b/a mm | 頭部に向って全左側毛 | | | | | | | 中央毛 | 頭部に向って全右側毛 | | | | | 生皮の各深度より | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 毛束形成小型毛(主に下毛) | | | | 副側上毛 | 副側上毛 | 左側上毛 | 暗黄上毛 | 右側上毛 | 毛束形成小型毛(主に下毛) | | | | 左側偽毛束形成小型毛 | 右側偽毛束形成小型毛 | 偽毛束形成小型毛(主に下毛) | 毛群の毛量 |
| | | | 偽毛束数(7) | | | | | | | | | 偽毛束数(2) | | | | | | | |
| | | | 棍状毛 | | 発育毛 | | | | | | | 発育毛 | | 棍状毛 | | | | | |
| | | | 無色毛根 | 有色毛根 | 青毛 | 幼毛 | 棍状毛 b/a | 青毛 b/a | 幼毛 b/a | 幼毛 b/a | 棍上毛 b/a | 幼毛 | 青毛 | 有色毛根 | 無色毛根 | | | | |
| 1 | 皮面 | 0.28/0.80 | 15 | 12 | 16 | 3 | 20.7/33.3 | 24.3/27.9 | 31.5/60.3 | 65.7/155.7 | 27.9/36.9 | 0 | 8 | 6 | 6 | 6 | 20 | 66 | 71 |
| 2 | 脂腺 0.2 | 0.30/0.80 | 20 | 7 | 16 | 3 | 18.9/31.5 | 24.3/27.9 | 31.5/58.5 | 67.1/153.7 | 27.9/36.9 | 0 | 8 | 5 | 7 | 46 | 20 | 66 | 71 |
| 3 | 脂腺 | 0.32/0.76 | 25 | 2 | 16 | 3 | 18.9/31.5 | 26.1/27.9 | 33.3/60.3 | 60.3/153.9 | 27.9/35.1 | 0 | 8 | 3 | 9 | 46 | 20 | 66 | 71 |
| 4 | 脂腺 0.4 | 0.28/0.76 | 25 | 2 | 16 | 3 | 18.9/22.5 | 24.3/27.9 | 31.5/60.3 | 60.3/153.9 | 33.3/35.1 | 0 | 8 | 2 | 10 | 46 | 20 | 66 | 71 |
| 5 | | 0.32/0.76 | 25 | 0 | 16 | 3 | 星状 17.1 | 22.5/27.9 | 31.5/58.6 | 62.1/148.5 | 星状 33.3 | 0 | 8 | 1 | 星状 11 | 44 | 20 | 64 | 70 |
| 6 | 0.7 | 0.28/0.68 | 9 | 0 | 16 | 4 | 星状 17.1 | 24.3/27.9 | 33.3/62.1 | 63.9/143.1 | 星状 27.9 | 0 | 8 | 1 | 星状 7 | 29 | 16 | 45 | 50 |
| 7 | | 0.28/0.68 | 0 | 0 | 16 | 4 | 星状 15.3 | 24.3/27.9 | 33.3/63.9 | 63.9/143.1 | 星状 24.3 | 0 | 8 | 0 | 星状 5 | 24 | 13 | 37 | 42 |
| 8 | 1.0 | 0.28/0.60 | 0 | 0 | 16 | 3.2 | 0 | 24.3/27.9 | 35.1/63.9 | 63.9/143.1 | 痕跡 | 1 | 8 | 0 | 0 | 21 | 9 | 30 | 33 |
| 9 | 1.3 | 0.32/0.52 | 0 | 0 | 16 | 3 | 0 | 27.9 | 53.1/63.9 | 89.1/143.1 | 痕跡 | 0 | 8 | 0 | 0 | 19 | 8 | 27 | 30 |
| 10 | 1.8 下毛球下底<br>2.0 上毛球下底 | 生皮の皮厚 2.2 mm | | | | | | | | | | | | | | | | | |

第34表　左側毛束の内訳

| 切片順序 | 毛幹幅 (μ) | 棍状毛根 Club hair root 有色 | 棍状毛根 Club hair root 無色 | 発育毛 Growing hair 幼毛 発育部位 毛葉 | 発育毛 Growing hair 青色 発育部位 毛柄 | 備考 |
|---|---|---|---|---|---|---|
| | 4.5 | | | 1 | | 偽毛束形成 |
| | 8.1 | | | 1 | 1 | 6個 |
| | 9.9 | | | 1 | 2 | root＋stalk＋blade 毛根＋毛柄＋毛葉 |
| | 11.7 | 5 | 10 | | 5 | 5＋5　＝10 |
| | 13.5 | 6 | 5 | | 4 | 3＋2　＝ 5 |
| 1 | 15.3 | 1 | | | 2 | 6＋3＋2＝11 |
| | 17.1 | | | | | 4＋2＋1＝ 7 |
| | 18.9 | | | | | 6＋2　＝ 8 |
| | 20.7 | | | | | 3＋2　＝ 5 |
| | | 12 | 15 | 3 | 16 | 46 |
| | | 27 | | 19 | | 46 |
| | 4.5 | | | 1 | | 対生毛 8 対 |
| | 8.1 | | | 1 | | 出現（分離） |
| | 9.9 | 3 | 12 | | 1 | |
| | 11.7 | 4 | 8 | 1 | 2 | |
| | 13.5 | | | | 4 | 皮脂腺位 |
| 2 | 15.3 | | | | 4 | Sebaceous glands region |
| | 17.1 | | | | 3 | |
| | 18.9 | | | | | |
| | 20.7 | | | | 2 | |
| | | 7 | 20 | 3 | 16 | |
| | | 27 | | 19 | | 46 |
| | 8.1 | | | 2 | | |
| | 9.9 | 1 | 14 | | 1 | 対生毛 8 対 |
| | 11.7 | 1 | 10 | 1 | 1 | |
| | 13.5 | | 1 | | 7 | 単生成毛11 |
| 3 | 15.3 | | | | 4 | 単生青毛 3 |
| | 17.1 | | | | 1 | 単生青毛或は幼毛16 |
| | 18.9 | | | | 1 | |
| | 20.7 | | | | 1 | 皮脂腺位 |
| | | 2 | 25 | 3 | 16 | |
| | | 27 | | 19 | | 46 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | 8.1 | | | 2 | | 単生成毛11 |
| | 9.9 | 1 | 16 | | | (SO) |
| | 11.7 | 1 | 8 | 1 | 4 | 単生青毛3 |
| | 13.5 | | 1 | | 5 | (SY) |
| | 15.3 | | | | 5 | 対生発育毛16 |
| | 17.1 | | | | | (POY or POC) |
| | 18.9 | | | | | |
| | 20.7 | | | | 2 | 皮脂腺位 |
| | | 2 | 25 | 3 | 16 | |
| | | 27 | | 19 | | |
| 5 | 9.9 | | 毛根痕跡 | 3 | 1 | |
| | 11.7 | | 〃 | | 2 | 棍状毛2消失 |
| | 13.5 | | | | 4 | |
| | 15.3 | | | | 6 | 対生毛解体 |
| | 17.1 | | | | 1 | |
| | 18.9 | | | | 1 | |
| | 20.7 | | | | 1 | |
| | | | 25 | 3 | 16 | |
| | | 25 | | 19 | | |
| 6 | 2.7 | | | 1 | | 毛芽(鞘毛) |
| | 9.9 | | 痕跡 | 1 | | 1個増 |
| | 11.7 | | 痕跡 | 2 | 3 | 無色毛根は星状或は無定形となる。 |
| | 13.5 | | | | 3 | |
| | 15.3 | | | | 5 | |
| | 17.1 | | | | 2 | |
| | 18.9 | | | | 1 | |
| | 20.7 | | | | 1 | |
| | 22.5 | | | | 1 | |
| | | | 9 | 4 | 16 | |
| | | 9 | | 20 | | 29 |
| 7 | 4.5 | | | 1 | | |
| | 9.9 | | 痕跡 | 1 | | 棍状毛(成毛) |
| | 11.7 | | 痕跡 | 2 | 4 | 5個消失 |
| | 13.5 | | | | 2 | |
| | 15.3 | | | | 5 | |
| | 17.1 | | | | 3 | |
| | 18.9 | | | | 1 | |
| | 20.7 | | | | | |
| | 22.5 | | | | 1 | |
| | | | 4 | 4 | 16 | |
| | | 4 | | 20 | | 24 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 11.7 | | | 1 | 1 | 幼毛2個増 |
| | 13.5 | | | 1 | 4 | 濃染した輪形 |
| | 15.3 | | | 1 | 6 | 毛球切片 |
| | 17.1 | | | 毛球 (bulbs) | 2 | |
| | 18.9 | | | 2 | 2 | 総棍状毛消失す |
| | 20.7 | | | | | |
| | 22.5 | | | | 1 | |
| | | | | 5 | 16 | |
| | | | | | | 21 |
| 9 | 13.5 | | | 1 | | |
| | 15.3 | | | 2 | 2 | 濃染した |
| | 17.1 | | | | 10 | 幼毛球消失す |
| | 18.9 | | | | 1 | |
| | 20.7 | | | | 1 | |
| | 22.5 | | | | 1 | |
| | 24.3 | | | | 1 | |
| | | | | 3 | 16 | |
| | | | | | | 19 |

第35表　右側毛束の内訳

| 切片順序 | 毛幹幅 (μ) | 棍状毛根 Club hair root 有色 | 棍状毛根 Club hair root 無色 | 発育毛 Growing hair 幼毛 発育部位 毛葉 | 発育毛 Growing hair 青色 発育部位 毛柄 | 備考 |
|---|---|---|---|---|---|---|
| 1 | 9.9 | 2 | 2 | | | |
| | 11.7 | 1 | 4 | | 1 | 毛束形成 |
| | 13.5 | 2 | | | 2 | 2個 |
| | 15.3 | 1 | | | 2 | 毛束内容 |
| | 17.1 | | | | 3 | 毛根＋毛柄 |
| | 18.9 | | | | | 6＋3＝ 9 |
| | 20.7 | | | | | 6＋5＝11 |
| | 22.5 | | | | | |
| | 24.3 | | | | | |
| | | 6 | 6 | | 8 | |
| | | 12 | | 8 | | 20 |
| 2 | 9.9 | 1 | 5 | | | 対毛1対分離 |
| | 11.7 | 1 | 2 | | 1 | その他は毛束形成 |
| | 13.5 | 2 | | | | |
| | 15.3 | 1 | | | 3 | |
| | 17.1 | | | | 1 | |
| | 18.9 | | | | 2 | |
| | 26.1 | | | | 1 | |
| | | 5 | 7 | | 8 | |
| | | 12 | | 8 | | 20 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 9.9 | 1 | 5 | | | 対毛7対分離 |
| | 11.7 | 1 | 3 | | 2 | 単生棍状毛5 |
| | 13.5 | | 1 | | 1 | 単生幼毛1 |
| | 15.3 | 1 | | | 3 | 分離 |
| | 17.1 | | | | | |
| | 18.9 | | | | 1 | 皮脂腺位 |
| | 24.3 | | | | 1 | |
| | | 3 | 9 | | 8 | |
| | | 12 | | 8 | | 20 |
| 4 | 9.9 | 1 | 7 | | | |
| | 11.7 | | 2 | | 1 | 対毛7対 |
| | 13.5 | | 1 | | 2 | |
| | 15.3 | 1 | | | 3 | |
| | 17.1 | | | | 1 | 皮脂腺位 |
| | 18.9 | | | | | |
| | 24.3 | | | | 1 | |
| | | 2 | 10 | | 8 | |
| | | 12 | | 8 | | 20 |
| 5 | 9.9 | | 8 | | | |
| | 11.7 | | 2 | | | 顕著な変化なし |
| | 13.5 | 1 | 1 | | 3 | |
| | 15.3 | | | | 3 | |
| | 17.1 | | | | | |
| | 18.9 | | | | 1 | |
| | 24.3 | | | | 1 | |
| | | 1 | 11 | | 8 | |
| | | 12 | | 8 | | 20 |
| 6 | 9.9 | | 5 | | | |
| | 11.7 | | 1 | | | 対毛解体し |
| | 13.5 | 1 | 1 | | 4 | 8棍状毛残留 |
| | 15.3 | | | | 2 | |
| | 17.1 | | | | | |
| | 18.9 | | | | 1 | |
| | 26.1 | | | | 1 | |
| | | 1 | 7 | | 8 | |
| | | 8 | | 8 | | 16 |
| 7 | 9.9 | | 2 | | | |
| | 11.7 | | 2 | | | 棍状毛根 |
| | 13.5 | 1 | | | 3 | 5個残留 |
| | 15.3 | | | | 2 | |
| | 17.1 | | | | 2 | |
| | 26.1 | | | | 1 | |
| | | 1 | 4 | | 8 | |
| | | 5 | | 8 | | 13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 15.3 | | | | 5 | 毛芽（鞘毛）の毛球切片1個あり |
| | 17.1 | | | | | |
| | 18.9 | | | | 1 | |
| | 20.7 | | | | 1 | |
| | 26.1 | | | | 1 | |
| | | | | 1 | 8 | 9 |
| 9 | 15.3 | | | | 1 | |
| | 17.1 | | | | 4 | 棍状毛根 |
| | 18.9 | | | | 1 | 凡て消失す |
| | 20.7 | | | | 1 | |
| | 22.5 | | | | 1 | |
| | 26.1 | | | | | |
| | | | | | 8 | 8 |

第36表　連続切片による背毛群の観察

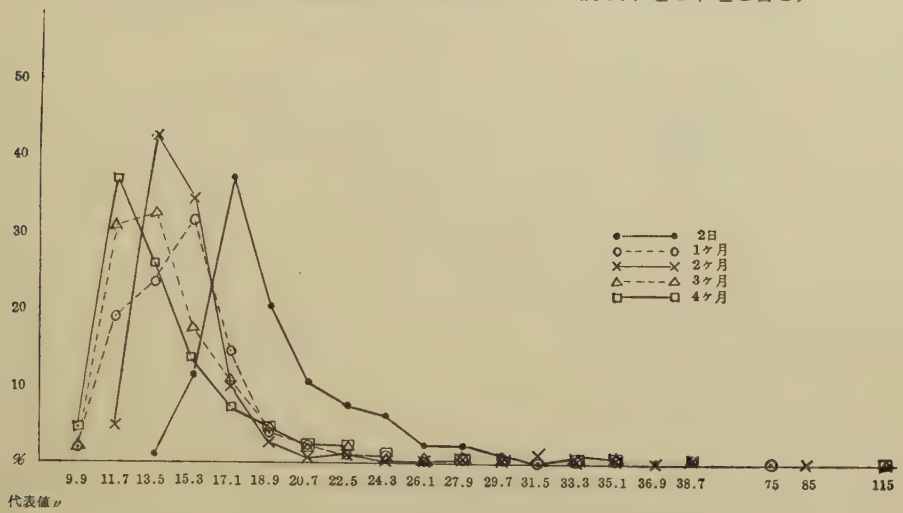
Animal no. N—12

| 順序と深度 | 頭部に向って全左側毛 毛幹幅(μ) | 棍状毛 | 発育毛 | 内訳 | 中央毛 棍状毛 毛根 | 頭部に向って全右側毛 毛幹幅(μ) | 棍状毛 | 発育毛 | 内訳 |
|---|---|---|---|---|---|---|---|---|---|
| 1. 皮面に接した切片 | 8.1 | 2 | 2 | 偽毛束2個 | | 8.1 | 7 | | 偽毛束3個 |
| | 9.9 | 10 | 1 | | b μ | 9.9 | 12 | 2 | |
| | 11.7 | 1 | 1 | 棍状毛根＋発育毛 | a μ | 11.7 | 6 | 2 | 棍状毛根＋発育毛 |
| | 13.5 | 1 | 5 | (1) 6 ＋ 5 ＝ 11 | 35.1 | 13.5 | 2 | 3 | (1) 9 ＋ 4 ＝ 13 |
| | 15.3 | 1 | | (2) 9 ＋ 5 ＝ 14 | 38.7 | 15.3 | 1 | 2 | (2) 10 ＋ 3 ＝ 13 |
| | 17.1 | | 1 | 毛束中に上毛なく凡て下毛である. | | 17.1 | | 1 | (3) 9 ＋ 5 ＝ 14 |
| | 18.9 | | | | | 18.9 | | 1 | 毛束中に上毛なく凡て下毛である. |
| | 20.7 | | | | 楕円形 | 20.7 | | 1 | |
| | | 15 | 10 | | | | 28 | 12 | |
| | | 25 | | | | | 40 | | |
| 2. 0.15 ↓ 0.4 mm | 6.3 | | | | | 6.3 | | 1 | |
| | 8.1 | 3 | 1 | 皮脂腺位 | | 8.1 | 3 | | 皮脂腺位 |
| | 9.9 | 9 | 2 | | | 9.9 | 16 | 2 | |
| | 11.7 | 1 | | 偽毛束の解体. | | 11.7 | 9 | 4 | 偽毛束解体. |
| | 13.5 | 1 | 2 | (1) 11毛の偽毛束は | 同 | 13.5 | 1 | 1 | (1) 13毛の偽毛束は |
| | 15.3 | 1 | 4 | 対生毛束4. P. O. Y., PO. C. | | 15.3 | | 2 | 対生毛束3. PO. Y., PO. C., |
| | 17.1 | | | 連生毛束1. TO. O. Y., | | 17.1 | | | 対生毛束2. PO. O., |
| | 18.9 | | 1 | TO. O. C. に解体す. | 上 | 18.9 | | 2 | 連生毛束1. TO. O. Y., TO. O. C |
| | 24.3 | | | (2) 14毛の偽毛束は | | 24.3 | | 1 | 単生棍状毛1. S. O. |
| | | 15 | 10 | 対生毛3. PO. Y., PO. C. | | | 29 | 13 | (2) 13毛の偽毛束は 対生毛束4. PO. Y., PO. C. 対生毛束3. PO. O., |
| | | 25 | | 連生毛2. TO. O. Y., TO. O. C. | | | 42 | | (3) 14毛の偽毛束は 対生毛束5. PO. Y, PO. C., 対生毛束2. PO. O., |
| | | | | 対生毛1. P. O. O., | | | | | 13毛束の偽毛束は下底より毛芽2個現わる. |

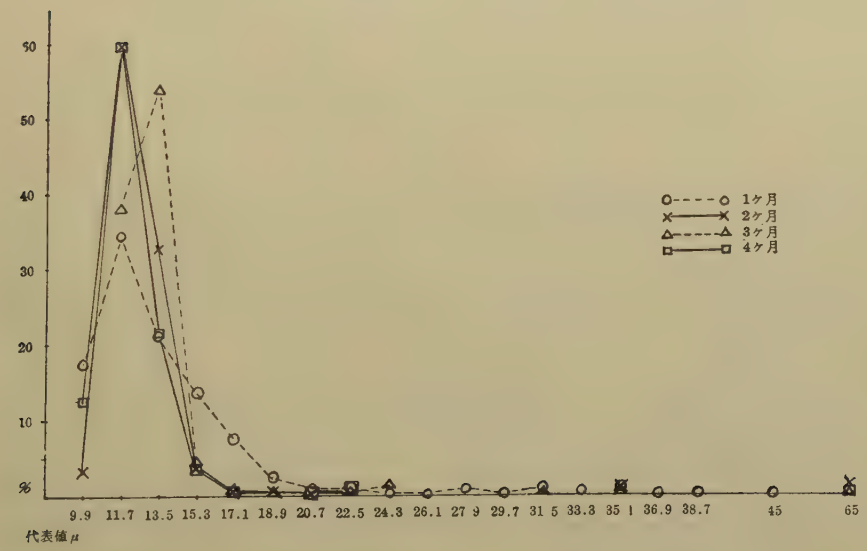
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.7 | | | | | 2.7 | | 2 | |
| | 4.5 | | | 皮脂腺消失. | 35.1 | 4.5 | | 1 | 皮脂腺消失. |
| 3. | 6.3 | | | | 35.1 | 6.3 | | | |
| 0.4 | 8.1 | | | 棍状毛は凡て消失. | | 8.1 | | 1 | 棍状毛1個残留し，毛芽（鞘 |
| ↓ | 9.9 | | 2 | | | 9.9 | 1 | 1 | 毛）3個出現す. |
| 0.7 | 11.7 | | 4 | | | 11.7 | | 4 | |
| mm | 13.5 | | 2 | | 星 状 | 13.5 | | 1 | |
| | 15.3 | | 1 | | | 15.3 | | 2 | |
| | 17.1 | | | | | 17.1 | | 1 | |
| | 18.9 | | 1 | | | 18.9 | | | |
| | 20.7 | | | | | 20.7 | | 1 | |
| | 22.5 | | | | | 22.5 | | | |
| | 24.3 | | | | | 24.3 | | 1 | |
| | | | 10 | | | | 1 | 15 | 16 |
| | 2.7 | | 1 | →鞘毛出現1個. | | 2.7 | | 1 | |
| | 4.5 | | | | | 4.5 | | 1 | |
| 4. | 6.3 | | | | 無定形 | 6.3 | | 1 | |
| | 8.1 | | | | | 8.1 | | | |
| | 9.9 | | 2 | | | 9.9 | | 3 | |
| | 11.7 | | 4 | →1個は皮質の色素顆粒が減 | | 11.7 | 1 | 1 | ヘマトキシリン濃染の毛球切 |
| | 13.5 | | 3 | 少し，髄質は萎縮している | | 13.5 | | 3 | 片出現. |
| | 15.3 | | | が，発育毛と認める. | | 15.3 | | 2 | 鞘毛の毛球と認める. |
| | 17.1 | | | | | 17.1 | | 1 | |
| | 18.9 | | 1 | | | 18.9 | | 1 | |
| | 20.7 | | | | | 20.7 | | | |
| | 22.5 | | | | | 22.5 | | | |
| | 24.3 | | | | | 24.3 | | 1 | |
| | | | 11 | | | | 1 | 15 | 16＋鞘毛球 1＝17 |
| | 9.9 | | 1 | 無色棍状毛根出現，これは上 | | 9.9 | | | 上方の切片で先端を見せてい |
| | 11.7 | 1 | 2 | 方の切片では発育毛として取 | 中央毛 | 11.7 | | 2 | た2個の鞘毛は濃染した |
| 5. | 13.5 | | 3 | り扱われていたものである. | 消失，下方に | 13.5 | | 1 | 外根鞘に取り巻かれた2個の |
| 1.0 | 15.3 | | 2 | | 球栓毛 | 15.3 | | 5 | 黒色毛球と成った. |
| ↓ | 17.1 | | | | 出現. | 17.1 | | 1 | |
| 1.5 | 18.9 | | 1 | | | 18.9 | | | |
| mm | 20.7 | | | | | 20.7 | | 1 | |
| | 22.5 | | | | | 22.5 | | | |
| | 24.3 | | | | | 24.3 | | 1 | |
| | 26.1 | | | | | 26.1 | | | |
| | | 1 | 9 | | | | | 11 | |

| | 棍状毛 | 発育毛 | |
|---|---|---|---|
| 6. 1.5 mm | | 1 | 19 | 最早毛群は毛包の下底に近く，その毛群の両端の外は両側毛の区別は出来ない. 発育の毛球上位切片19個，棍状毛根1個残留. |
| 7. | | | 16 | 発育毛の毛球切片16個残留. |
| 8. 1.66 mm | | | 2 | 毛球2個あり，これらは発育中の青毛の毛包で，充分に伸びつくしたもので，最早下方に移動しない. |

31図　KN，背毛，小型暗葉毛の百分率度数分布図（分岐下毛と下毛を含む）




32図，K. N. 腹毛，小型暗葉毛の百分率度数分布図（月別比較）




4.　生毛から成獣の被毛まで

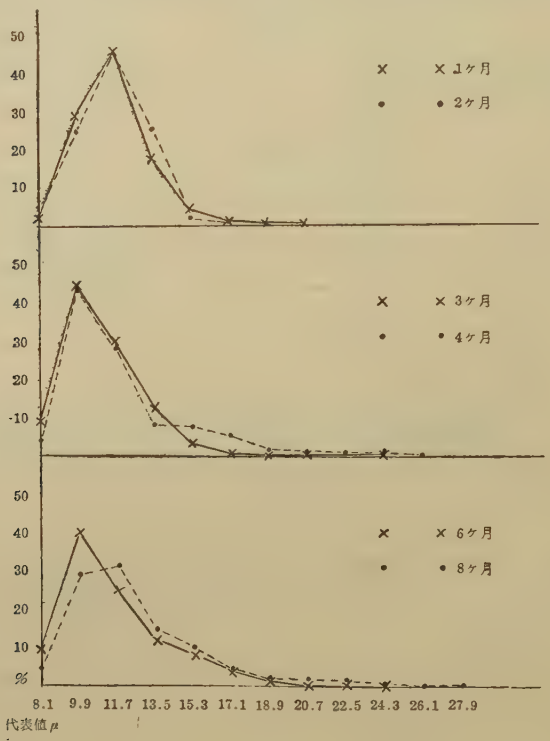
Nutria の3ケ月末期の胎児の背部中央では既に生毛群の毛原基は完全に発生している．

中央毛と両側毛を含む3原毛群（original trio group）はほとんど球桿期，鞘毛期で大型のものは芒先を表わして幼毛期である．

それらの間隙の後方にある副側毛の多くは毛芽期である．

出生直後までの約1.5ケ月（懐胎期間，gestation period，を135日—137日として生後2日をいう）に大型毛群中の副側毛，小型毛群の側毛を代表する小型，中型の明葉毛及暗葉毛（分岐毛）は毛柄部まで成長した青毛である．又小型（暗葉）副側毛は毛葉下位，毛頸まで成長

33図 K. N. V—1→8背毛，小型暗葉毛の百分率度数分布図（月別比較）




した幼毛である（第56. 57表. 第36図）. 胎児期の3ケ月末の3原毛群の誕毛期より約2.5ケ月を経た生後1ケ月では少数の大型毛を除く他，多数の生毛は完熟した成毛でL切片によると成毛の毛包下位には後継毛の毛芽及び球桿毛を，R切片では成毛包に接して2次副側毛の毛原基の兆を示す Hämatoxylin に濃染した細胞塊が認められる．又稀れに2次副側毛の幼毛が生毛の青毛と相接して偽毛束の一歩をふみ出したものもある．以上により生後1ケ月の資料では $G_1$ の anagen の中期より $G_2$ のanagen の初期なども示しているが，概して生後1ケ月間は生毛（ウブゲ）（$G_1$; birth coat）の成熟期間とみられる．

(1) 下毛の2次発生と換毛

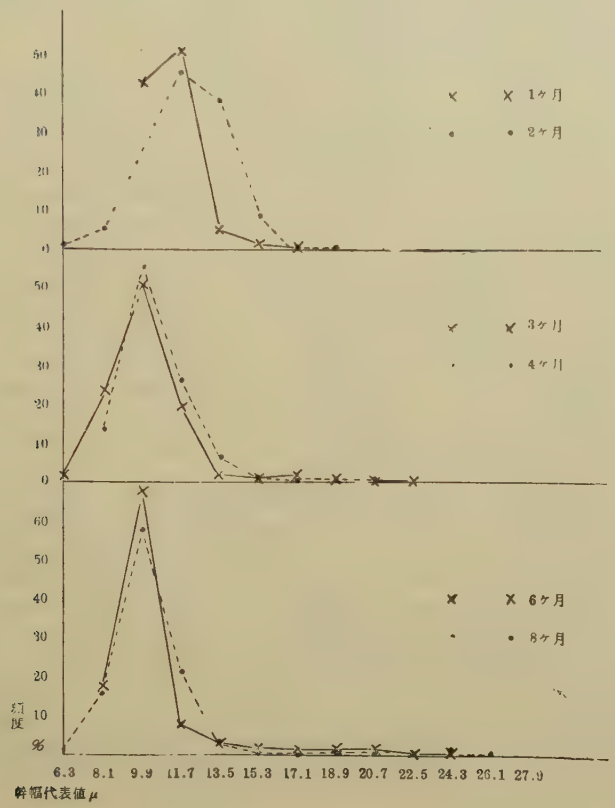
生後2ケ月の被毛（pelage）では多くの成毛が発育中の後継毛と同じ毛管内に或は同毛孔内に1対か或は数個が混生して真毛束，偽毛束を形成して換毛の態勢にあるが，これらの後継毛の多くは発育盛りの青毛に他ならない．

なお生後2ケ月の毛叢では下毛的な小型毛で成毛，青毛，幼毛の各種の独立した単生毛があり，又表皮面では同毛孔内にあっても皮脂腺の上位まで毛束を切り下げて解体すると明らかに他の毛包から発生する単生毛の場合もある．これらは換毛による後継毛ではなくて2次発生の小型副側毛で下毛の代表である．

生後2ケ月の総副側毛は毛包を100％として138.46％であるるが，この内生毛の毛包は38.49％で生毛の棍状毛は全部残留し，それと同数38.46％の発育毛（青毛＋幼毛）と対生している（第64表，第39図）．

2次副側毛は既に成毛，青毛，幼毛を混えて61.6％が発生し生毛の毛包の約160％である．

34図　KNV 腹毛，小型暗茶毛の百分率度数分布図，月別比較




生後3ケ月の資料（寒冷季資料Ⓡ切片，1月15日屠殺処分）では生毛は凡て残留し，又第2毛世代（$G_2$）も成毛と青毛の状態で生毛包と同数が存在し，総毛包の夫々25%宛となつている．

なお2次副側毛は総副側毛の75%で総副側生毛数に対しては300%の増加となつている．

青毛と幼毛を2ケ月－3ケ月，即ち3ケ月目に生じた副側毛とせば約40%の増となる．

生後4ケ月の資料（寒冷季資料Ⓡ切片，2月15屠殺処分）では生毛（$G_1$）総毛包の14.5%で，第2毛世代（$G_2$）は総毛包の20.2%で，この $G_2$ の数が丁度生毛の毛包数に等しいことになり，生毛は総毛包に対して20.2%－14.5%＝5.7% 落毛していることになる．又2次副側毛包は総毛包の79.8%で生毛包数の（79.8÷20.2）×100＝395%である．

即ち生後4ケ月間に増加した毛包数は395%である．4ケ月目に増加した青毛＋幼毛の毛包数は約15%である．

以上5例は1腹の幼獣の資料で10月より2月までの寒冷季に亘っている．次に生後8ケ月の資料は，生後3月より11月下旬にかけて得たので採毛資料は温暖季を経過しており，毛皮の資料Ⓡ切片は11月20日採取で寒冷季である．この毛皮のⓇ切片によれば前記同様に未だ副側毛包の増加が確認される．

即ち生毛に対する増加率は不明であるが幼毛の単生毛のみで総毛包の5.8%で，これは寒

冷季であることと同時に幼毛期に対する成毛が落毛することはほとんど無いので，2次発生の副側毛と考えてよい，又検鏡によれば明らかに新生の幼毛である．更に生後12ケ月以上の動物について検鏡の結果，新生包の発生は全く発見されない．

毛包の増加は副側毛（下毛）のみであるが，生後2ケ月までに最も多く増加して副側毛の生毛に対して16%，2ケ月→3ケ月の間で約4%，3ケ月→4ケ月の間で15%，8ケ月目では上述のように明白でないがまだ多数の増加を示している．例えば1毛群の生毛包数を6或は7とせば成獣の1毛群の平均毛包は45→46で生毛包の約600−700%である．

Nutria は生後約12ケ月間に1毛群の毛包は500→600%増加することになる．なお生後3ケ月では副側毛の後継毛は $G_3$ が総毛包の0.4%で，生毛包の2.4%が $TG_1O, G_2O, G_3C$ として連生している．生後4ケ月では副側毛の $TG_1O, G_2O, G_3C$ の $G_3C$ と，$PG_2O$, $G_3$（Y+C）で $G_3$（Y+C）の合計は総毛包の7.6%で，生毛包に対する約37.6%を生じている．又生後3ケ月では2次副側毛 $g_1$ の後継毛は $Pg_1O, g_2$，$Pg_1O_1g_2C$ として $g_2Y$ と $g_2C$ の合計は総毛包の33.1%を生じている．4ケ月では $Pg_1O, g_2$（Y+C）として $g_2$（Y+C）を総毛包に対して35.8%生じている（第39図）．

KNY−1→8（飼育幼獣）の背腹両下毛の柄幅の頻度曲線図より観るに，生後1ケ月，2ケ月の頻度曲線は最頻数の頂点は同じで，生毛の成毛の継続を示し，2ケ月と3ケ月の間で生毛の落毛，換毛が推定される3ケ月，4ケ月，6ケ月では2次副側毛（$g_1^{1-n}$）の新毛の追補と生毛の副側毛（$G_{2-n}$）との換毛により1階級下位を持続している（第33.34図）．

以上は生毛の下毛的な暗葉毛が（生毛の副側毛）下級，同級，上級のものと種々変化に富んだ換毛をするが，その内小型のもの程同級の後継毛を生ずる場合が多くやや大型のものは多く小型に換毛する傾向があるからである．

Nutria の生毛（$G_1$）の下毛には Dry[11]（1926）の mouse における step-up と step-down と現状態維持の3つの傾向がある．

但し $G_2$ 以後の後継毛（$G_{3-n}$）は凡て step-up のみに変じて，8ケ月では生毛（$G_1$）と同じ階級の最頻数に復しているが，ちらばりの幅は広くなって曲線の脚は上位の階級に伸びている．

KNV−1→8は春夏秋冬に亘る採毛資料によるので換毛が早期に行われていて，生毛及び初期の棍状毛の残留は少いので頻度は毛世代の変異をやや明確に表わしているものと受け取れる（第33.34図）．以上と同様に生後数ケ月間を KN. O→KN. 4 の資料で観察するに，一腹の幼獣から得た数字で，別個体であるので明確な結果ではないが，上記と略同様の傾向はうかがえる（第31図，第32図）．

これは10月より2月までの5ケ月で秋季より厳冬に及び，成毛の交換脱落は僅少であるので KNV−1→8 のように明らかな変化を示さないことがうなづかれる．即ち生毛の副側毛（$G_1$）に当る．大型下毛が残留しているので月別下毛の曲線は図のように小型下毛が増量してはじめて4ケ月の曲線で頂点が一級下位に現われている．以上は成獣被毛の寒冷季と温暖季との比較調査の結果より判断出来ることである．

第37表　毛叢（上毛＋下毛）の度数分布表とその分岐表

Animal no. K. N. 1. 背毛

| 1.8 μ 級間 | | | | 10. μ 級間 | | | | 観察総数に対する | | 小型毛の毛葉部，明，暗比率 | | 下毛，分岐毛，上毛の分離 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 階級 μ | | 代表値 μ | 頻度 | 階級 μ | | 代表値 μ | 頻度 | 級間 11.8μ % | 級間 10 μ % | 暗葉 % | 明葉 % | 下毛と分岐下毛 | 分岐上毛と上毛 | | 下毛と上分岐下毛の頻度% |
| 5.4 | 7.2 | 6.3 | | | | | | | | | | | | | |
| 7.2 | 9.0 | 8.1 | | 0 | 10 | 5 | | | | | | | | | |
| 9.0 | 10.8 | 9.9 | 20 | | | | | 1.9+ | | 100 | 0 | ↑ | 20 | | 2.1− |
| 10.8 | 12.6 | 11.7 | 195 | | | | | 18.9− | | 100 | 0 | | 195 | | 20.1+ |
| 12.6 | 14.4 | 13.5 | 242 | | | | | 23.4+ | | 100 | 0 | 下 | 242 | | 25.0• |
| 14.4 | 16.2 | 15.3 | 326 | | | | | 31.5+ | | 100 | 0 | 毛 | 326 | | 33.7− |
| 16.2 | 18.0 | 17.1 | 149 | | | | | 14.4+ | | 100 | 0 | | 149 | | 15.4− |
| 18.0 | 19.8 | 18.9 | 40 | 10 | 20 | 15 | 972 | 3.9− | 94.0+ | 73− | 27+ | 分 | 29 | 11 | 3.0− |
| 19.8 | 21.6 | 20.7 | 20 | | | | | 1.9+ | | 30− | 70+ | 岐 | 6 | 14 | 0.6+ |
| 21.6 | 23.4 | 22.5 | 4 | | | | | 0.4− | | 11+ | 89− | 下毛 | 1 | 3 | 0.1+ |
| 23.4 | 25.2 | 24.3 | 4 | | | | | 0.4− | | 0 | 100 | | | 4 | |
| 25.2 | 27.0 | 26.1 | 2 | | | | | 0.2− | | | | | | 2 | |
| 27.0 | 28.8 | 27.9 | 1 | 20 | 30 | 25 | 31 | 0.1− | 3.0− | | | | | 1 | |
| 28.8 | 30.6 | 29.7 | 3 | | | | | 0.3− | | | | | | 3 | |
| 30.6 | 32.4 | 31.5 | 2 | | | | | 0.2− | | | | | | 2 | |
| 32.4 | 34.2 | 33.3 | 1 | | | | | 0.1− | | | | | | 1 | |
| 34.2 | 36.0 | 35.1 | 3 | | | | | 0.3− | | | | | | 3 | |
| 35.0 | 37.8 | 36.9 | 1 | | | | | 0.1− | | | | | | 1 | |
| 37.8 | 39.0 | 38.7 | 2 | 30 | 40 | 35 | 12 | 0.2− | 1.2− | | | | | 2 | |
| | | | | 40 | 50 | 45 | 6 | | 0.6− | | | | | 6 | |
| | | | | 50 | 60 | 55 | 10 | | 1.0− | | | | | 10 | |
| | | | | 60 | 70 | 65 | 2 | | 0.2− | | | | | 2 | |
| | | | | 70 | 80 | 75 | 1 | | 0.1− | | | | | 1 | |
| | | | | | | | (S) 1034 | | (S) 100.1 % | | | | (S) 968 | (S) 66 | 100% |
| | | | | | | | 100% | | | | | | 94− % | 6+ % | |

分岐上毛↓

第38表　下毛＋分岐下毛の頻度率（%）比較表（1）

| 動物記号 / 柄幅代表値 | 背毛 | | | |
|---|---|---|---|---|
| | K. N. 1 % | K. N. 2 % | K. N. 3 % | K. N. 4 % |
| 8.1 μ | | | | |
| 9.9 | 2.1− | | 1.7− | 5 + |
| 11.7 | 20.1+ | 5.9− | 32.5− | 42.4− |
| 13.5 | 25.0− | 55.0• | 34.1+ | 29.9− |
| 15.3 | 33.7− | 32.2− | 18.5− | 15.7− |
| 17.1 | 15.4− | 6.5+ | 6.1+ | 5.4+ |
| 18.9 | 3.0− | 0.4+ | 4.2+ | 1.5− |
| 20.7 | 0.6+ | | 1.6− | 0.2+ |
| 22.5 | 0.1+ | | 1.1+ | |
| 24.3 | | | 0.1+ | |
| 26.1 | | | 0.1+ | |
| S % | 100 | 100 | 100 | 100.1 |

| | 腹 | | 毛 | |
|---|---|---|---|---|
| 8.1 | | | | |
| 9.9 | 19.5+ | 3.3+ | | 12.7+ |
| 11.7 | 38.0+ | 60.1+ | 38.7− | 60.8+ |
| 13.5 | 23.3− | 32.9+ | 55.2+ | 22.1− |
| 15.3 | 14.7+ | 3.3+ | 4.1− | 3.5+ |
| 17.1 | 3.4+ | 0 | 0.3− | 0.6+ |
| 18.9 | 1.0+ | 0.3+ | 0.8− | 0 |
| 20.7 | | | 0.3− | 0.2+ |
| 22.5 | | | 0.3− | |
| 24.3 | | | 0.5+ | |
| S % | 99.9 | 99.9 | 100.2 | 99.9 |

第39表 下毛＋分岐下毛の頻度率(%)比較表(2)

| 動物記号 / 柄幅代表値 | 背毛 K. N. V-1. % | K. N. V-2. % | K. N. V-3. % | K. N. V-4. % | K. N. V-6. % | K. N. V-8. % |
|---|---|---|---|---|---|---|
| 8.1 | 2.0+ | 2.6+ | 8.7+ | 3.9− | 9.4+ | 4.3− |
| 9.9 | 28.8+ | 24.4+ | 44.6+ | 43.1+ | 40.0+ | 28.9− |
| 11.7 | 45.8+ | 45.1+ | 30.0• | 28.1• | 24.5+ | 31.5+ |
| 13.5 | 17.4− | 24.9+ | 12.0− | 8.1− | 11.9+ | 14.8+ |
| 15.3 | 4.3+ | 2.2+ | 3.2+ | 7.7+ | 8.0+ | 9.8− |
| 17.1 | 1.1− | 0.6− | 0.6− | 5.4− | 3.7− | 4.3− |
| 18.9 | 0.4+ | | 0.1+ | 1.4+ | 1.3− | 2.2+ |
| 20.7 | 0.1+ | | 0.4+ | 1.1− | 0.4− | 2.0+ |
| 22.5 | | | 0.1+ | 0.5− | 0.6+ | 1.6+ |
| 24.3 | | | 0 | 0.2+ | 0.1+ | 0.2+ |
| 26.1 | | | 0.1+ | | | 0.2+ |
| 27.9 | | | | | | 0.2+ |
| S % | 99.9 | 99.8 | 99.8 | 100 | 99.9 | 100 |
| | 腹毛 | | | | | |
| 6.3 | | 0.8+ | 1.7− | | | 0.2− |
| 8.1 | | 5.2+ | 23.3+ | 10.3− | 17.1+ | 16.0− |
| 9.9 | 42.3− | 45.3+ | 51.0− | 55.3− | 67.4+ | 57.8− |
| 11.7 | 50.9+ | 39.0− | 19.0− | 26.3+ | 8.3− | 21.4+ |
| 13.5 | 4.9+ | 8.8+ | 1.4+ | 6.2+ | 2.6− | 3.1+ |
| 15.3 | 1.5+ | 0.6− | 0.7+ | 1.2− | 1.7+ | 0.4− |
| 17.1 | 0.4− | 0.3− | 1.4+ | 0.2+ | 1.1+ | 0 |
| 18.9 | | | 0.7+ | 0 | 0.9− | 0.8− |
| 20.7 | | | 0.5− | 0.5− | 0.6− | 0.2− |
| 22.5 | | | 0.2+ | | 0 | 0 |
| 24.3 | | | | | 0.3− | 0 |
| 26.1 | | | | | | 0.2− |
| S % | 100 | 99.9 | 99.9 | 100 | 100 | 100.1 |

(2) 分岐域の換毛

一腹の幼獣（KNO—4）の別個体からの採毛の比較では明らかでないが，飼育幼獣 KN (V—1→8) の月別連続採毛によって観察するに暗葉毛は図のように明らかに各階級で葉幅を増し逓進して所謂 step-up を示している（第17図）.

明葉毛では，生毛（$G_1$）と後継毛（$G_{2-n}$）との柄幅の同級のものを比較すると，後継毛の葉幅の方が生毛の葉幅より狭いので step-down ではないかと錯覚を起すが，これはあくまで毛包を異にする者同志の比較であって，2ケ月幼獣（KN. 2）の資料のR切片の観察と採毛の全毛との比較対照によれば次表のように同毛包から発生した後継毛は常に生毛より拡大していて step-up している．なお図によると明葉毛はやや後退しているが.（第17図）これは下位の階級を放棄して上位についているので step-up の結果である．更に暗葉毛は高度に step-up している結果分岐域は拡張している（第 9, 10, 11, 12 図）.

分岐域と下毛の観察（生後2ケ月資料）

第40表 (a) 分岐域の生毛（調査表より）

| 生毛の全毛 | 分岐域の根幅 μ | 柄　幅 μ | 葉　幅 μ | |
|---|---|---|---|---|
| | | | 暗 | 明 |
| | 9.9 ～ 11.7 | 15.3 | 19.18 | 23.79 |
| | 11.7 ～ 13.5 | 17.1 | 20.70 | 25.45 |
| | 13.5 | 18.9 | 21.60 | 31.31 |

第41表 (b) 先代毛（生毛）と後継毛との比較（背毛群R切片の観察）

| 対生毛 | 9.9 | | | | 11.7 | | | |
|---|---|---|---|---|---|---|---|---|
| 先代毛の根幅 (μ)→ | 暗葉 | | 明葉 | | 暗葉 | | 明葉 | |
| 後継毛の葉幅 (μ)→と数 | 幅 | 数 | 幅 | 数 | 幅 | 数 | 幅 | 数 |
| | 17.1 | 1 | 24.3 | 1 | 18.9 | 1 | 26.2 | 2 |
| | 18.9 | 4 | 26.1 | 1 | 20.7 | 1 | 29.7 | 1 |
| | 20.7 | 1 | | | 24.3 | 1 | | |
| | 22.5 | 2 | | | 26.1 | 2 | | |
| | 24.3 | 2 | | | 27.9 | 3 | | |
| | | 10 | | 2 | | 8 | | 3 |
| | 平均 | 20.7 μ | | 25.2 μ | | 24.3 μ | | 27.3 μ |

生後2ケ月のR切片で7個毛束型の毛群の多くは側毛は明葉，暗葉が両側に対面していてこれらは凡て先代毛と対生して上表のような関係を持っている（b）．又採毛資料による棍状毛の全毛（club-hair of whole length）の調査によれば生毛の分岐域において全毛の各部位の平均値は（a）表のようである．この（a），（b）の2表を対照すると，R切片の調査による先代毛の根幅と全毛調査による棍状毛の根幅の測定値は同級のもので．このR切片の先代毛は明らかに生毛の分岐毛である．さらにそれらの後継毛である幼毛は上記のように分岐毛を生ずることを物語っている．さらに葉幅は明葉，暗葉ともに後継毛はやや毛幅が拡大して僅かに type step-up の兆がある．

(3) 上毛の換毛

KNV—1→8の上毛の葉幅曲線図（第35図）で観るに，1ケ月，2ケ月は類似の曲線である．これは生毛の完成期の存続を示し，3ケ月，4ケ月，5ケ月に次代（$G_2$）の完成期を，5ケ月，6ケ月，7ケ月に$G_3$の完成期を，6ケ月，7ケ月，8ケ月に$G_4$の完成期を示している．R切片で観察のように$G_1 \rightarrow G_2$は飛躍的に拡大するが（第58, 59, 60表），換毛によって現われた小型上毛の後継毛（$G_2$）と生毛（$G_1$）の同階級を比較するとその葉幅は$G_1$より$G_2$が狭いが，交代毛は常に柄幅を逓増し，階級が進むに従って葉幅は拡張して$G_1 < G_2 < G_3 < G_4$で，明らかに type step-up を表わしている．

飼育幼獣の背部上毛の葉幅曲線

明葉毛の分岐上毛から暗葉毛の第1上毛に亘る全上毛の一連の柄幅に対する葉幅の関係をFISHERの多項式回帰の計算によることにした．飼育観察した動物の1ケ月から8ケ月までの月別の資料の観察値の個数は第25表のように調査された結果8個→11個であるので観察値を分析するための直交多項式は統計数値表（統計科学研究会篇）によった．

(1) 1ケ月幼獣（K. N. Y—1）を例にとれば次のようである。

第42表

| 柄幅級間10 μ 代表値 μ | 15 | 25 | 35 | 45 | 55 | 65 | 75 | 85 | 95 | S (y) | $\bar{y}=a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| x | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | |
| 葉幅観察値平均 $Y_\mu$ | 24.22 | 36.78 | 62.10 | 90.22 | 93.60 | 102.30 | 108.90 | 124.35 | 126.80 | 769.27 | 85.47 |

毛の柄幅を級間10 μに分って葉幅の各観察値はその級に含まれるものの平均値を求めて当てた．次は観察値が9個の場合を分析するための直交多項式である．

第 43 表

| x | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | S ($\xi^2$) | 乗数 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\xi_1$ | −4 | −3 | −2 | −1 | 0 | 1 | 2 | 3 | 4 | 60 | 1 |
| $\xi_2$ | 28 | 7 | −8 | −17 | −20 | −17 | −8 | 7 | 28 | 2772 | 3 |
| $\xi_3$ | −14 | 6 | 13 | 9 | 0 | −9 | −13 | −6 | 14 | 990 | $\frac{5}{6}$ |
| $\xi_4$ | 14 | −21 | −11 | 9 | 18 | 9 | −11 | −21 | 14 | 2002 | $\frac{7}{12}$ |
| $\xi_5$ | −4 | 11 | −4 | −9 | 0 | 9 | 4 | −11 | 4 | 468 | $\frac{3}{20}$ |

回帰方程式 $Y=a+b_1(x-\bar{x})+b_2(x-\bar{x})^2+b_3(x-\bar{x})^3+b_4(x-\bar{x})^4+b_5(x-\bar{x})^5$

の係数 $b_1$, $b_2$, $b_3$, ……を計算するかわりに方程式 $Y=a+b'_1\xi_1+b'_2\xi_2+b'_3\xi_3+b'_4\xi_4+b'_5\xi_5$

の係数 $b'_1$, $b'_2$, $b'_3$…等を求めるのである．

$$b'_1=\frac{S(\xi_1 y)}{S(\xi_1{}^2)},\quad b'_2=\frac{S(\xi_2 y)}{S(\xi_2{}^2)},\quad b'_3=\frac{S(\xi_3 y)}{S(\xi_3{}^2)},\quad b'_4=\frac{S(\xi_4 y)}{S(\xi^2{}_4)},\quad b'_5=\frac{S(\xi_5 y)}{S(\xi_5{}^2)}.$$

次に上表，式などを組合せて次のようにとく．

$S(\xi_1 y)=-96.88-110.34-124.2-90.22+102.3+217.8+373.05+507.2=778.71$

$S(\xi_2 y)=-1156.37$

$S(\xi_3 y) = 193.58$

$S(\xi_4 y) = 267.03$

$S(\xi_5 y) = -263.45$

$b'_1 = \frac{778.71}{60} \fallingdotseq 12.98_{(-)}$

$b'_2 = \frac{-1156.37}{2772} \fallingdotseq -0.42_{(-)}$

$b'_3 = \frac{193.58}{990} \fallingdotseq 0.20_{(-)}$

$b'_4 = \frac{267.03}{2002} \fallingdotseq 0.13_{(+)}$

$b'_5 = \frac{-263.45}{468} \fallingdotseq -0.56_{(+)}$

第44表　上毛の葉幅曲線を表わす期待値

| Y= | 1次直線 | | 2次抛物線 | | 3次曲線 | | 4次曲線 | | 5次曲線 | | 観察値 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $a+b'_1\xi_1$ | 期待値 $\mu$ | $+b'_2\xi_2$ | 期待値 $\mu$ | $+b'_3\xi_3$ | 期待値 $\mu$ | $+b'_4\xi_4$ | 期待値 $\mu$ | $+b'_5\xi_5$ | 期待値 $\mu$ | $\mu$ |
| $Y_1$ | 86.47−51.92 | 33.55 | −11.76 | 21.79 | −2.8 | 24.59 | +1.82 | 26.41 | +2.24 | 28.65 | 24.22 |
| $Y_2$ | 〃 −38.94 | 46.53 | − 2.94 | 43.59 | +1.2 | 44.79 | −2.73 | 42.06 | −6.16 | 35.90 | 36.78 |
| $Y_3$ | 〃 −25.96 | 59.51 | + 3.36 | 62.87 | +2.6 | 65.47 | −1.43 | 64.04 | +2.24 | 66.28 | 62.10 |
| $Y_4$ | 〃 −12.98 | 72.49 | + 7.14 | 79.63 | +1.8 | 81.43 | +1.17 | 82.60 | +5.04 | 87.64 | 90.22 |
| $Y_5$ | 〃 + 0 | 85.47 | + 8.40 | 93.87 | 0 | 93.87 | +2.34 | 96.21 | 0 | 96.21 | 93.60 |
| $Y_6$ | 〃 +12.98 | 98.72 | + 7.14 | 105.86 | −1.8 | 104.06 | +1.17 | 105.23 | −5.04 | 100.19 | 102.30 |
| $Y_7$ | 〃 +25.96 | 111.70 | + 3.36 | 115.06 | −2.6 | 112.46 | −1.43 | 111.03 | −2.24 | 108.79 | 108.90 |
| $Y_8$ | 〃 +38.94 | 124.68 | − 2.94 | 121.74 | −1.2 | 120.54 | −2.73 | 117.81 | +6.16 | 123.97 | 124.35 |
| $Y_9$ | 〃 +51.92 | 137.66 | −11.76 | 125.90 | +2.8 | 128.70 | +1.82 | 130.52 | −2.24 | 128.28 | 126.80 |

第45表　観察値（Y）の分散分析の平方和の直交成分えの分割

（+），（−）は4捨5入を示す．

| 同帰の項目 | $S(\xi^2)$ | $S(\xi y)$ | $b'$ | 平方和 | $b'$の自由度 | 残差平方和 | 残差の自由度 | 残差平均平方和 | t | 確率 | 評定 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1次$\xi_1$ | 60 | 778.71 | $12.98^{(-)}$ | 10107.6558 | 1 | 701.4761 | 7 | 100.2108 | 10.04 | 極めて小 | 極めて有意 |
| 2次$\xi_2$ | 2772 | −1156.37 | $-0.42^{(-)}$ | 485.6754 | 1 | 215.8007 | 6 | 35.9667 | 3.67 | 0.02～0.01 | 有意 |
| 3次$\xi_3$ | 990 | 193.58 | $0.20^{(-)}$ | 38.7160 | 1 | 177.0847 | 5 | 35.4169 | 1.04 | 0.50～0.30 | 適 |
| 4次$\xi_4$ | 2002 | 267.03 | $0.13^{(+)}$ | 34.7139 | 1 | 142.3708 | 4 | 35.5927 | 0.98 | 0.50～0.30 | 不適 |
| 5次$\xi_5$ | 468 | −263.45 | $-0.56^{(+)}$ | 147.5320 | 1 | 29.5527 | 3 | 9.8509 | 3.86 | 0.05～0.02 | 有意 |

観察値（y）の平方和 $= S(y-y)^2 = S(\bar{y})^2 - \frac{S^2(y)}{N}$

$= 76562.0577 - 65752.9258$

$= 10809.1319$

（これより1次$\xi_1$の平方和を引く）

5次は有意であるが蛇行している，4次も同様であるので，3次の曲線を採用することにする。

(2) 生後2ケ月幼獣 (K. N. V—2) の上毛曲線

第46表 上毛の葉幅曲線を表わす期待値

| Y= | 1次直線 a+b′1 ξ1 | 期待値 μ | 2次抛物線 +b′2 ξ2 | 期待値 μ | 3次曲線 +b′3 ξ3 | 期待値 μ | 4次曲線 +b′4 ξ4 | 期待値 μ | 観察値 μ |
|---|---|---|---|---|---|---|---|---|---|
| Y1 | 7901−49.28 | 29.73 | − 12.25 | 17.50 | + 2.59 | 20.09 | + 3.29 | 23.38 | 23.73 |
| Y2 | 〃 −35.20 | 43.81 | − 1.75 | 42.06 | + 1.85 | 43.91 | − 6.11 | 37.80 | 33.53 |
| Y3 | 〃 −21.12 | 57.89 | + 5.25 | 63.14 | − 2.59 | 60.55 | − 1.41 | 59.14 | 61.10 |
| Y4 | 〃 − 7.04 | 71.97 | + 8.75 | 80.72 | + 1.11 | 81.83 | + 4.23 | 86.06 | 79.30 |
| Y5 | 〃 + 7.04 | 86.05 | + 8.75 | 94.80 | − 1.11 | 93.69 | + 4.23 | 97.92 | 105.90 |
| Y6 | 〃 +21.12 | 100.13 | + 5.25 | 105.38 | + 2.59 | 107.97 | − 1.41 | 106.56 | 101.25 |
| Y7 | 〃 +35.20 | 114.21 | − 1.75 | 112.56 | + 1.85 | 114.41 | − 6.11 | 108.31 | 110.70 |
| Y8 | 〃 +49.28 | 128.29 | − 12.25 | 116.04 | − 2.59 | 113.45 | + 3.29 | 116.74 | 116.55 |

第47表 観察値 (y) の分散分析の平方和の直交成分えの分割 (+), (−)は4捨5入

| 回帰の項目 | S(ξ²) | S (ξy) | b′ | 平方和 | b′の自由度 | 残差平方和 | 残差の自由度 | 残差平均平方和 | t | 確率 | 評定 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1次 ξ1 | 168 | 1182.64 | 7.04(−) | 8325.7856 | 1 | 751.2049 | 6 | 125.2008 | 8.15 | 極めて小 | 極めて有意 |
| 2次 ξ2 | 168 | −286.86 | − 1.71(−) | 490.5306 | 1 | 260.6743 | 5 | 52.1348 | 3.06 | 0.05〜0.02 | 有意 |
| 3次 ξ3 | 264 | − 96.96 | − 0.37(−) | 35.8752 | 1 | 224.7991 | 4 | 56.1997 | 0.79 | 0.50〜0.30 | 適 |
| 4次 ξ4 | 616 | 286.72 | 0.47(−) | 134.7584 | 1 | 90.0407 | 3 | 30.0135 | 2.11 | 0.20〜0.10 | 不適 |

(3) 生後3ケ月幼獣 (K. N. V—3) の上毛曲線

第48表 上毛の葉幅曲線を表わす期待値

| Y= | 1次直線 a+b′1ξ1 | 期待値μ | 2次抛物線 +b′2ξ2 | 期待値μ | 3次曲線 +b′3ξ3 | 期待値μ | 4次曲線 +b′4ξ4 | 期待値μ | 観察値μ |
|---|---|---|---|---|---|---|---|---|---|
| Y1 | 6509−47.28 | 17.81 | +0.65 | 18.46 | +4.71 | 23.17 | −0.09 | 23.08 | 22.62 |
| Y2 | 〃 −31.52 | 33.57 | 0 | 33.57 | −4.71 | 28.86 | +0.21 | 29.07 | 30.78 |
| Y3 | 〃 −15.76 | 49.33 | −0.39 | 48.94 | −4.71 | 44.23 | −0.03 | 44.20 | 42.26 |
| Y4 | 〃 0 | 65.09 | −0.52 | 64.57 | 0 | 64.57 | −0.18 | 64.39 | 63.94 |
| Y5 | 〃 +15.76 | 80.85 | −0.39 | 80.46 | +4.71 | 85.17 | −0.03 | 85.14 | 87.75 |
| Y6 | 〃 +31.52 | 96.61 | 0 | 96.61 | +4.71 | 101.32 | +0.21 | 101.53 | 99.60 |
| Y7 | 〃 +47.28 | 112.37 | +0.65 | 113.02 | −4.71 | 108.31 | −0.09 | 108.22 | 108.70 |

第49表 観察値 (y) の分散分析の平方和の直交成分えの分割 (+), (−) は4捨5入

| 回帰の項目 | S(ξ²) | S(ξy) | b′ | 平方和 | b′の自由度 | 残差平方和 | 残差の自由度 | 残差平均平方和 | t | 確率 | 評定 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1次ξ1 | 28 | 441.37 | 15.76 | 6955.9912 | 1 | 154.4461 | 5 | 30.8892 | 15.0 | 極めて小 | 極めて有意 |
| 2次ξ2 | 84 | 10.81 | 0.13 | 1.4053 | 1 | 153.0408 | 4 | 30.6081 | 0.20 | 0.90〜0.80 | 不適 |
| 3次ξ3 | 6 | −28.23 | −4.71 | 132.9633 | 1 | 20.0775 | 3 | 6.6925 | 4.45 | 0.05〜0.02 | 有意 |
| 4次ξ4 | 154 | −5.05 | −0.03 | 0.1515 | 1 | 19.9260 | 2 | 9.9630 | 0.12 | 0.90 以上 極めて大 | 適 |

(4) 生後4ケ月幼獣 (K. N. V—4) の上毛曲線

第50表　上毛の葉幅曲線を表わす期待値

| Y= | 1次直線 | | 2次抛物線 | | 3次曲線 | | 4次曲線 | | 観察値μ |
|---|---|---|---|---|---|---|---|---|---|
| | a+b′1ξ1 | 期待値μ | +b′2ξ2 | 期待値μ | +b′3ξ3 | 期待値μ | +b′4ξ4 | 期待値μ | |
| Y1 | 79.9−61.67 | 18.23 | −5.53 | 12.70 | +9.24 | 21.94 | +0.56 | 22.50 | 21.68 |
| Y2 | 〃 −44.05 | 35.85 | −0.79 | 35.06 | −6.60 | 28.46 | −1.04 | 27.42 | 30.21 |
| Y3 | 〃 −26.43 | 53.47 | +2.37 | 55.84 | −9.24 | 46.60 | −0.24 | 46.36 | 44.45 |
| Y4 | 〃 − 8.81 | 71.09 | +3.95 | 75.04 | −3.96 | 71.08 | +0.72 | 71.80 | 69.20 |
| Y5 | 〃 + 8.81 | 88.71 | +3.95 | 92.66 | +3.96 | 96.62 | +0.72 | 97.34 | 100.60 |
| Y6 | 〃 +26.43 | 106.33 | +2.37 | 108.70 | +9.24 | 117.94 | −0.24 | 117.70 | 118.30 |
| Y7 | 〃 +44.05 | 123.95 | −0.79 | 123.16 | +6.60 | 129.76 | −1.04 | 128.72 | 126.65 |
| Y8 | 〃 +61.67 | 141.57 | −5.53 | 136.04 | −9.24 | 126.80 | +0.56 | 127.36 | 128.07 |

第51表　観察値 (y) の分散分析の平方和の直交成分えの分割　(+), (−) は4捨5入

| 回帰の項目 | S(ξ²) | S(ξy) | b′ | 平方和 | b′の自由度 | 残差平方和 | 残差の自由度 | 残差平均平方和 | t | 確率 | 評定 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1次ξ1 | 168 | 1479.88 | 8.81(−) | 13037.7428 | 1 | 601.0754 | 6 | 100.1792 | 11.41(−) | 極めて小 | 極めて有意 |
| 2次ξ2 | 168 | −132.14 | −0.79(−) | 104.3906 | 1 | 496.6848 | 5 | 99.3370 | 1.03(−) | 0.50～0.30 | 不適 |
| 3次ξ3 | 264 | −348.62 | −1.32(+) | 460.1784 | 1 | 36.5064 | 4 | 9.1266 | 7.1(+) | 0.01~0.001 | 有意 |
| 4次ξ4 | 616 | 49.02 | +0.08(−) | 3.9216 | 1 | 32.5848 | 3 | 10.8616 | 0.6(+) | 0.70～0.50 | 適 |

(5) 生後6ケ月幼獣 (K. N. V—6) の上毛曲線

第52表　上毛の葉幅曲線を表わす期待値

| Y= | 1次直線 | | 2次抛物線 | | 3次曲線 | | 4次曲線 | | 5次曲線 | | 観察値μ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | a+b′1ξ1 | 期待値μ | +b′2ξ2 | 期待値μ | +b′3ξ3 | 期待値μ | +b′4ξ4 | 期待値μ | +b′5ξ5 | 期待値μ | |
| Y1 | 99.82−61.35 | 38.47 | −25.05 | 13.42 | +5.40 | 18.82 | +5.04 | 23.86 | +1.53 | 25.39 | 25.11 |
| Y2 | 〃 −49.08 | 50.74 | −10.02 | 40.72 | −1.08 | 39.64 | −5.04 | 34.60 | −3.06 | 31.54 | 32.67 |
| Y3 | 〃 −36.81 | 63.01 | + 1.67 | 64.68 | −3.96 | 60.72 | −5.04 | 55.68 | −0.51 | 55.17 | 54.95 |
| Y4 | 〃 −24.54 | 75.28 | +10.02 | 85.29 | −4.14 | 81.15 | −0.84 | 80.31 | +2.04 | 82.35 | 77.70 |
| Y5 | 〃 −12.27 | 87.55 | +15.03 | 102.58 | −2.52 | 100.06 | +3.36 | 103.42 | +2.04 | 105.46 | 113.99 |
| Y6 | 〃 0 | 99.82 | +16.70 | 116.52 | 0 | 116.52 | +5.04 | 121.56 | 0 | 121.56 | 115.37 |
| Y7 | 〃 +12.27 | 112.09 | +15.03 | 127.12 | +2.52 | 129.64 | +3.36 | 133.00 | −2.04 | 130.96 | 131.98 |
| Y8 | 〃 +24.54 | 124.36 | +10.02 | 134.38 | +4.14 | 138.52 | −0.84 | 137.68 | −2.04 | 135.64 | 137.25 |
| Y9 | 〃 +36.81 | 136.63 | + 1.67 | 138.30 | +3.96 | 142.26 | −5.04 | 137.22 | +0.51 | 137.73 | 136.16 |
| Y10 | 〃 +49.08 | 148.90 | −10.02 | 138.70 | +1.08 | 139.78 | −5.04 | 134.74 | +3.06 | 137.80 | 138.70 |
| Y11 | 〃 +61.35 | 161.17 | −25.05 | 136.12 | −5.40 | 130.72 | +5.04 | 135.76 | −1.53 | 134.23 | 134.10 |

第53表　観察値 (y) の分散分析の平方和の直交成分えの分割　(+), (−) は4捨5入

| 回帰の項目 | S(ξ²) | S(ξy) | b′ | 平方和 | b′の自由度 | 残差平方和 | 残差の自由度 | 残差平均平方和 | t | 確率 | 評定 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1次ξ1 | 110 | 1349.79 | 12.27(+) | 16561.9233 | 1 | 2916.6248 | 9 | 324.0694 | 7.14 | 極めて小 | 極めて有意 |
| 2次ξ2 | 858 | −1431.87 | −1.67 | 2391.2229 | 1 | 525.4019 | 8 | 65.6752 | 6.03 | 極めて小 | 極めて有意 |
| 3次ξ3 | 4290 | −774.61 | −0.18 | 139.4298 | 1 | 385.9721 | 7 | 55.1388 | 1.23 | 0.30～0.20 | 不適 |
| 4次ξ4 | 286 | 241.53 | 0.84 | 202.8852 | 1 | 183.0869 | 6 | 30.5144 | 2.57 | 0.05～0.02 | 有意 |
| 5次ξ5 | 156 | −80.26 | −0.51 | 40.9326 | 1 | 142.1543 | 5 | 28.4308 | 1.19 | 0.50～0.25 | 適 |

(6) 生後8ケ月幼獣 (K. N. V−8) の上毛曲線

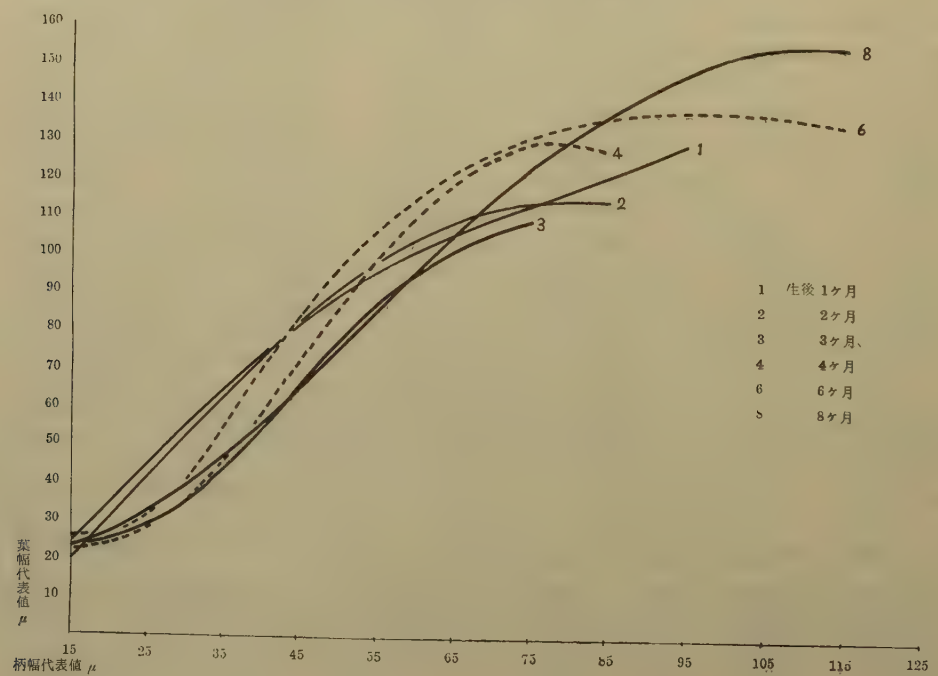
第54表 上毛の葉幅曲線を表わす期待値

| Y= | 1次直線 | | 2次抛物線 | | 3次曲線 | | 4次曲線 | | 観察値μ |
|---|---|---|---|---|---|---|---|---|---|
| | a+b′1ξ1 | 期待値μ | +b′2ξ2 | 期待値μ | +b′3ξ3 | 期待値μ | +b′4ξ4 | 期待値μ | |
| Y1 | 97.49−74.0 | 23.49 | −9.0 | 14.49 | +8.4 | 22.89 | +0.6 | 23.49 | 23.40 |
| Y2 | 〃 −59.2 | 38.29 | −3.6 | 34.69 | −1.68 | 33.01 | −0.6 | 32.41 | 32.86 |
| Y3 | 〃 −44.4 | 53.09 | +0.6 | 53.69 | −6.16 | 47.53 | −0.6 | 46.93 | 45.90 |
| Y4 | 〃 −29.6 | 67.89 | +3.6 | 71.49 | −6.44 | 65.05 | +0.1 | 65.15 | 67.50 |
| Y5 | 〃 −14.8 | 82.69 | +5.4 | 88.09 | −3.92 | 84.17 | +0.4 | 84.57 | 81.90 |
| Y6 | 〃 0 | 97.49 | +6.0 | 103.49 | 0 | 103.49 | +0.6 | 104.09 | 105.30 |
| Y7 | 〃 +14.8 | 112.29 | +5.4 | 117.69 | +3.92 | 121.61 | +0.4 | 122.01 | 117.90 |
| Y8 | 〃 +29.6 | 127.09 | +3.6 | 130.69 | +6.44 | 137.13 | −0.1 | 137.03 | 143.10 |
| Y9 | 〃 +44.4 | 141.89 | +0.6 | 142.49 | +6.16 | 148.65 | −0.6 | 148.05 | 146.70 |
| Y10 | 〃 +59.2 | 156.69 | −3.6 | 153.09 | +1.68 | 154.77 | −0.6 | 154.17 | 152.10 |
| Y11 | 〃 +74.0 | 171.49 | −9.0 | 162.49 | −8.4 | 154.09 | +0.6 | 154.69 | 155.70 |

第55表 観察値 (y) の分散分析の平方和の直交成分えの分割 (+), (−) は4捨5入

| 回帰の項目 | (Sξ²) | S(ξy) | b′ | 平方和 | b′の自由度 | 残差平方和 | 残差の自由度 | 残差平均平方和 | t | 確率 | 評定 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1次ξ1 | 110 | 1628.06 | 14.8 | 24095.2880 | 1 | 725.1704 | 9 | 80.5744 | 17.29 | 極めて小 | 極めて有意 |
| 2次ξ2 | 858 | −511.14 | −0.60 | 306.6840 | 1 | 418.4864 | 8 | 52.3108 | 2.42 | 0.05～0.02 | 有意 |
| 3次ξ3 | 4290 | −1206.84 | −0.28 | 337.9152 | 1 | 80.5712 | 7 | 11.5101 | 5.41 | 0.001 | 有意 |
| 4次ξ4 | 286 | 29.64 | 0.10 | 2.9640 | 1 | 77.6072 | 6 | 12.9345 | 0.49 | 0.70～0.50 | 適 |

35図 上毛の毛葉幅と毛柄幅との関係図 (飼育獣, KNV1−8)



### 5. 毛世代 hair generation

Collin[9]（1923）は mouse で初期幼獣被毛（juvenal pelage），後期幼獣被毛（post juvenal pelage）を経て成獣被毛（adult pelage）に移るとし，1例では生後2.5ケ月，他の1例では4.5ケ月ではじめて幼獣被毛期より成獣被毛期に移る換毛が始められたとしている．Dry[12]（1928）は mouse の被毛の週期は32日で，17日−21日までに休止状態を整える．それで17日—32日までを休止期（telogen）と呼んでいる．従つて生後4ケ月経過後下毛の1毛包より毛世代順に $G_1$，$G_2$，$G_3$，$G_4$ の棍状毛の1毛束を述べ又 $G_3$ の成毛の成長が止まるまでに動物は成獣に近くなる．即ち mouse は約3ケ月で成獣化すると報じている．Nutira では生皮の切片及び採毛資料の調査成績を総括して，被毛の発生と換毛の週期を推定した．

なお生後2日，生後2ケ月（1部）の調査経過以外は第82，88表と同じ方法によるので掲載を省略する．即ち次のようである．

第56表　生後2日目幼獣の毛幹幅の頻度分布

動物記号 K. N. O.　（毛包の横断面を含む50毛群の検鏡調査成績より）

| 級間 1.8μ | | | | | | 級間 10.μ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 階級 μ | | 代表値μ | 頻度 | | | 階級 μ | | 代表値 μ | 頻度 | | |
| | | | 青毛（毛柄） | 幼毛（毛葉） | 計 | | | | 青毛（毛柄） | 幼毛（毛葉） | 計 |
| | 1.8 | 0.9 | （）内は白毛 | 2 | 2 | 40 | 50 | 45 | | 0 | 0 |
| 1.8 | 3.6 | 2.7 | | 8 | 8 | | | | | | |
| 3.6 | 5.4 | 4.5 | | 14 | 14 | 50 | 60 | 55 | | 1 | 1 |
| 5.4 | 7.2 | 6.3 | | 7 | 7 | | | | | | |
| 7.2 | 9.0 | 8.1 | | 14 | 14 | 60 | 70 | 65 | | 1 | 1 |
| 9.0 | 10.8 | 9.9 | | 35 | 35 | | | | | | |
| 10.8 | 12.6 | 11.7 | | 27 | 27 | 70 | 80 | 75 | | 2 | 2 |
| 12.6 | 14.4 | 13.5 | | 15 | 15 | | | | | | |
| 14.4 | 16.2 | 15.3 | 6 (1) | 13 | 19 | 80 | 90 | 85 | | 1 | 1 |
| 16.2 | 18.0 | 17.1 | 46 (6) | 11 | 57 | | | | | | |
| 18.0 | 19.8 | 18.9 | 27 (1) | 3 | 30 | 90 | 100 | 95 | | 1 | 1 |
| 19.8 | 21.6 | 20.7 | 18 | 3 | 21 | | | | | | |
| 21.6 | 23.4 | 22.5 | 13 | | 13 | 100 | 110 | 105 | | 2 | 2 |
| 23.4 | 25.2 | 24.3 | 8 | | 8 | | | | | | |
| 25.2 | 27.0 | 26.1 | 4 | | 4 | | | | | | |
| 27.0 | 28.8 | 27.9 | 2 | | 2 | | | | | | |
| 28.8 | 30.6 | 29.7 | 2 | | 2 | | | | | | |
| 30.6 | 32.4 | 31.5 | 1 | | 1 | | | | | | |
| 32.4 | 34.2 | 33.3 | 0 | | 0 | | | | | | |
| 34.2 | 36.0 | 35.1 | 2 | | 2 | | | | | | |
| 36.0 | 37.9 | 36.9 | 0 | | 0 | | | | | | |
| 37.8 | 39.6 | 38.7 | 1 | | 1 | | | | | | |
| | | | 130 | 152 | 282 | | | | | 8 | 8 |

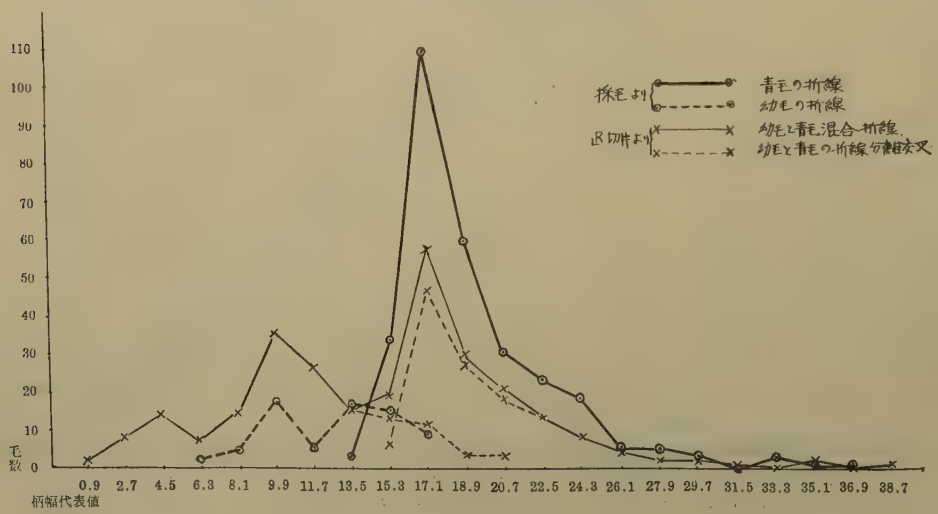
総毛包量　378　100 %　　青毛量　130　34.4%
幼毛量　160　42.3〃　　鞘毛量　88　23.3〃

第57表 生後2日目幼獣の毛幹幅の頻度分布

動物記号 K.N.O. (採毛の検鏡調査成績より)

| 階級間 1.8μ | | | | | | 階級間 10μ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 階級 μ | | 代表値μ | 頻度 | | | 階級 μ | | 代表値 μ | 頻度 | | |
| | | | 青毛(毛柄) | 幼毛(毛葉) | 計 | | | | 青毛(毛柄) | 幼毛(毛葉) | 計 |
| 5.4 | 7.2 | 6.3 | | 2 | 2 | 40 | 50 | 45 | | 4 | 4 |
| 7.2 | 9.0 | 8.1 | | 4 | 4 | | | | | | |
| 9.0 | 10.8 | 9.9 | | 17 | 17 | 50 | 60 | 55 | | 7 | 7 |
| 10.8 | 12.6 | 11.7 | | 5 | 5 | | | | | | |
| 12.6 | 14.4 | 13.5 | 3 | 16 | 19 | 60 | 70 | 65 | | 5 | 5 |
| 14.4 | 16.2 | 15.3 | 33 | 15 | 48 | | | | | | |
| 16.2 | 18.0 | 17.1 | 109 | 9 | 118 | 70 | 80 | 75 | | 7 | 7 |
| 18.0 | 19.8 | 18.9 | 59 | | 59 | | | | | | |
| 19.8 | 21.6 | 20.7 | 30 | | 30 | 80 | 90 | 85 | | 8 | 8 |
| 21.6 | 23.4 | 22.5 | 23 | | 23 | | | | | | |
| 23.4 | 25.2 | 24.3 | 18 | | 18 | 90 | 100 | 95 | | 8 | 8 |
| 25.2 | 27.0 | 26.1 | 5 | | 5 | | | | | | |
| 27.0 | 28.8 | 27.9 | 5 | | 5 | 100 | 110 | 105 | | 4 | 4 |
| 28.8 | 30.6 | 29.7 | 3 | | 3 | | | | | | |
| 30.6 | 32.4 | 31.5 | 0 | | 0 | 110 | 120 | 115 | | 3 | 3 |
| 32.4 | 34.2 | 33.3 | 3 | | 3 | | | | | | |
| 34.2 | 36.0 | 35.1 | 1 | | 1 | | | | | | |
| 36.0 | 37.8 | 36.9 | 1 | | 1 | | | | | | |
| 37.8 | 39.6 | 38.7 | 0 | | 0 | | | | | | |
| | | | 293 | 68 | 361 | | | | | 46 | 46 |

36図 2日目の被毛頻度分布



生後2日幼獣の被毛発生の状態

Ⓡ切片の観察．毛幹幅の代表値が 11.7 μ 以下では明らかに色素顆粒が多くて毛髄は不明瞭で鞘毛の毛芒，毛葉先端の断面を示している．又代表値が 13.5 μ 以上の多くは毛髄が鮮明で中心部にあり，皮質に色素顆粒がやや少く明らかに毛頸の下位から毛柄部を現わしている．その一部には色素多量で毛髄の位置は中心を外れて不明瞭で鞘毛の毛葉腹の断面を示しているものもある．幹幅代表値 11.7 μ 以下のものは代表値 9.9 μ を頂点とする度数折線を画き明らかに鞘毛の毛芒部を示している．

次に幹幅代表値 13.5 μ 以上のものは一部の鞘毛の毛葉腹と多くの測定標準位に達した毛柄部（hair stalk）の断面を示す等，幼毛青毛の一群は 17.1 μ を頂上として 26.1 μ 附近に急降下して 38.7 μ まで裾を引いている．以上の折線は 13.5 μ を交合点とする 2 頂曲線であるが，これは図のように明らかに幼毛，青毛の 2 つの曲線に分離しうる（第36図）．

剪毛の観察．なお被毛の剪毛の資料内で下毛的な細い繊維は 2—5 mm 程度のものが過半数を占めその他は 1 cm 未満の発育中の幼毛で，上毛的な粗大毛も 0.5—1.5 cm 未満の幼毛である．この小型毛の内で毛頸から下方の毛柄部を測定しうるのは 0.5 cm 以上で柄幅 13.5 μ（Ⓡ切片では確認出来ないが剪毛の測定値中にはある）の少数と 15.3 μ 以上のものに限られ，柄幅 11.7 μ 以下の小型毛を代表する青毛はない．更に毛葉部で色素を多量に含んでいて毛髄を明らかに透視出来ない発育不充分なものは 0.5 cm 以下で 17.1 μ 以下 6.3 μ までの各階級にわたつている．

11.7 μ 以下の小型幼毛を代表するものは特に採毛が困難なため度数が僅少であるが，9.9 μ ではやや増加しているのが前述の毛皮のⓇ切片の観察の一頂点と一致している．しかしながらこの内には小型毛の各階級の毛葉の先端が混在しているので特に暗葉幅の代表値が 9.9 μ を示すような級の小型毛の多量発生の将来を物語るとはいえない．しかるにⓇ切片の全資料にわたつて調査の結果，黄色明帯（agouti band）を示す毛葉部の切片は全く発見出来ないので，これらの多くは小型の下毛的な暗葉毛と見做してよい．以上のように柄幅 11.7 μ—17.1 μ を境として下毛的な暗葉の小型毛の発生をうかがいえよう．

又これより大型毛の粗毛の大部分は未だ発育中で（0.5—1.5 cm）測定標準位の毛柄部まで現われていないので，その測定部位は毛頸或は毛頸下位などで一定しないが，およその傾向は認められる．以上の考察から生後 2 日幼獣の被毛では上下毛の境界を思わせる明暗葉毛混合の小型の青毛と上毛的な幼毛とが被毛を代表していて，下毛的な暗葉の小型毛の発生は暗示的である．青毛34.4%，幼毛42.3%，鞘毛23.3%である．

第58表　生後2ケ月幼獣の毛群に含まれる新，旧毛の比較（中央毛の場合）

動物記号 K. N. 2. 背毛　　（ⓐ印は新毛の比較部位を示す）

| 毛群順序 | 対生毛束（R切片より） | | | | | | 全毛（抜取毛より） | | | | | 明葉(M)又は暗葉(A) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 先代($G_1$) | 後継毛($G_2$) | | | | | 先代毛($G_1$) | | | | | |
| | 毛根幅 | 幹幅 a | 幹幅 b | 幹幅 c | 色調その他 | 成長部位 | 根幅 | 柄幅 | 頸幅 | 葉幅 | 芒幅 | |
| 1 | 13.5 μ | 56.7 μ ⓐ | 33.3 μ | 29.7 μ | 暗褐灰色 | 毛頸 | 13.5 μ | 17.1～18.7 μ | 17.1 μ ⓐ 56.7 | 26.1～29.7 μ | — μ | (M)又は(A) ⓐは不明 |
| 2 | 〃 | 53.1 ⓐ | 29.7 | 27.9 | 〃〃〃 | 〃〃 | | | ⓐ 53.1 | | | ⓐは不明 |
| 3 | 〃 | 47.7 ⓐ | 31.5 | 26.1 | 暗黄色 | 葉明帯下位 | | | | ⓐ 47.7 | | ⓐは(M) |
| 4 | 15.3 | 103.5 ⓐ | 40.5 | 65.7 | 黒色 | 暗葉下位 | 15.3 | 20.7～23.4 | 17.1～20.7 | 35.1～40.5 ⓐ 103.5 | 22.5 | (M) ⓐは(A) |
| 5 | 〃 | 80.1 ⓐ | 36.9 | 38.7 | 暗黄色 | 葉明帯下位 | | | | ⓐ 80.1 | | ⓐは(M) |
| 6 | 〃 | 44.1 ⓐ | 31.5 | 15.3 | 黒色 | 毛芒 | | | | | ⓐ44.1 | ⓐは不明 |
| 7 | 〃 | 49.5 ⓐ | 35.1 | 27.9 | 暗褐灰色 | 毛頸 | | | ⓐ 49.5 | | | ⓐは不明 |
| 8 | 17.1 | 72.1 ⓐ | 35.1 | 38.7 | 暗褐灰色 | 毛頸 | 17.1 | 22.5 | 18.9 ⓐ 72.1 | 33.3 | — | (M) ⓐは不明 |
| 9 | 〃 | 62.1 ⓐ | 38.7 | 35.1 | 〃〃〃 | 〃〃 | | | ⓐ 62.1 | | | ⓐは不明 |
| 10 | 〃 | 76.5 ⓐ | 35.1 | 44.1 | 〃〃〃 | 〃〃 | | | ⓐ 76.5 | | | ⓐは不明 |
| 11 | a. 26.1 b. 24.3 | 130.5 ⓐ | 56.7 | 92.7 | 〃〃〃 | 暗毛葉 | 26.1 | 44.1 | 36.9 | 92.7 ⓐ 130.5 | — | (M) ⓐは(A) |
| 12 | a. 40.5 b. 33.3 | 123.3 ⓐ | 56.7 | 62.1 | 〃〃〃 | 〃〃〃 | 40.5 | 72.9～80.1 | 62.1～69.3 | 114.3～117.9 ⓐ 123.3 | | ⓐは(A) |
| 13 | a. 38.7 b. 38.7 | | | | 乳白色 | 後継毛のない棍状毛根 | | | | | | |
| 14 | a. 38.7 b. 38.7 | | | | 〃〃〃 | 〃〃〃 | | | | | | |
| 15 | 先代毛($G_1$)なし | 145.7 ⓐ | 56.7 | 92.7 | 暗褐灰色 | 暗葉毛の毛葉単生 | | | | ⓐ 146.7 | | ⓐは(A) |
| 16 | 〃〃〃 | 125.1 ⓐ | 49.5 | 74.7 | 〃〃〃 | 〃〃〃 | | | | ⓐ 125.1 | | ⓐは(A) |

第59表　生後2ケ月幼獣の毛群に含またる新，旧毛の比較（側毛の場合）

動物記号 K. N. 2. 背毛　　（ⓐ印は新毛の比較部位を示す）

| 毛群順序 | 対生毛束（Ⓡ切片より） 先代($G_1$) 毛根幅 | 後継毛($G_2$) 幹幅 a | b | c | 色調その他 | 成長部位 | 全毛（抜取毛より） 先代毛($G_1$) 根幅 | 柄幅 | 頸幅 | 葉幅 | 芒幅 | 明葉(M)又は暗葉(A) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | a, b 9.9 μ | 26.1 μ ⓐ | 26.1 μ | | 暗褐灰色 | 暗毛葉 | 9.9 μ | 11.7～13.5 μ | 11.7～13.5 μ | 15.3～17.1 μ ⓐ 26.1 | — μ | (A) ⓐは(A) |
| 2 | 〃 | 31.5 ⓐ | 31.5 | | 〃 | 〃 | | | | ⓐ 31.5 | | ⓐは(A) |
| 3 | a, b 11.7 | 26.1 ⓐ | 26.1 | | 〃 | 〃 | 11.7 | 15.3～17.1 | 15.3 | 22.5 ⓐ 26.1 | | ⓐは(A) |
| 4 | 〃 | 27.9 ⓐ | 27.9 | | 〃 | 〃 | | | | ⓐ 27.9 | | ⓐは(A) |
| 5 | 〃 | 29.7 ⓐ | 29.7 | | 〃 | 〃 | | | | ⓐ 29.7 | | ⓐは(A) |
| 6 | 〃 | 35.1 ⓐ | 35.1 | | 〃 | 〃 | | | | ⓐ 35.1 | | ⓐは(A) |
| 7 | 〃 | 26.1 ⓐ | 26.1 | | 〃 | 〃 | | | | ⓐ 26.1 | | ⓐは(A) |
| 8 | 〃 | 29.7 ⓐ | 29.7 | | 〃 | 〃 | | | | ⓐ 29.7 | | ⓐは(A) |
| 9 | 〃 | 27.9 ⓐ | 27.9 | | 〃 | 〃 | | | | ⓐ 27.9 | | ⓐは(A) |
| 10 | a, b 13.5 | 33.3 ⓐ | 33.3 | | 暗褐灰色 | 暗毛葉 | 13.5 | 17.1～18.9 | 17.1 | 26.1～29.7 ⓐ 33.3 | | ⓐは(A) |
| 11 | 〃 | 31.5 ⓐ | 31.5 | | 暗黄色 | 明毛葉下位 | | | | ⓐ 31.5 | | ⓐは(M) |
| 12 | a, b 15.3 | 65.7 ⓐ | 33.3 | 36.9 | 暗褐灰色 | 毛頸 | 15.3 | 20.7～22.5 | 17.1～20.7 ⓐ 65.7 | 35.1～40.5 | — | (M) ⓐは不明 |
| 13 | 先代毛($G_1$)脱落後 | 63.9 ⓐ | 35.1 | 35.1 | 暗褐灰色（単生毛） | 毛頸 | | | ⓐ 63.9 | | | ⓐは不明 |
| 14 | 〃 | 130.5 ⓐ | 51.3 | 74.7 | 〃 | 暗毛葉 | | | | ⓐ 130.5 | | ⓐは(A) |
| 15 | 〃 | 116.1 ⓐ | 42.3 | 62.1 | 〃 | 〃 | | | | ⓐ 116.1 | | ⓐは(A) |

第60表　生後2ケ月幼獣の腹毛群に含まれる新，旧毛の比較　（3原毛の場合）

動物記号 K. N. 2. 腹毛　　（ⓐ印は新毛の比較部位を示す）

| 毛群順序 | 対生毛束（ℝ切片より） | | | | | | 全毛（抜毛より） | | | | | 明葉(M)又は暗葉(A) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 先代($G_1$) | 後継毛($G_2$) | | | | | 先代毛($G_1$) | | | | | |
| | 毛根幅 | 幹幅 a | b | c | 色調その他 | 成長部位 | 根幅 | 柄幅 | 頸幅 | 葉幅 | 芒幅 | |
| 1 | a, b 9.9μ | 15.3μ ⓐ | 15.3μ | | 薄墨色 | 毛柄 | 9.9μ | 15.3～18.9μ ⓐ 15.3 | 13.5～17.1μ | 18.9～20.7μ | —μ | (M)又は(A) ⓐは不明 |
| 2 | 〃 | 18.9 ⓐ | 18.9 | | 〃 | 〃 | | ⓐ 18.9 | | | | ⓐは不明 |
| 3 | 〃 | 17.1 ⓐ | 17.1 | | 〃 | 〃 | | ⓐ 17.1 | | | | ⓐは不明 |
| 4 | 〃 | 18.9 ⓐ | 18.9 | | 〃 | 〃 | | ⓐ 18.9 | | | | ⓐは不明 |
| 5 | a, b 11.7 | 35.1 ⓐ | 29.7 | 20.7 | 〃 | 〃 | 11.7 | 17.1 ⓐ 35.1 | 15.3 | 20.7 | — | (M)又は(A) ⓐは不明 |
| 6 | 〃 | 35.1 ⓐ | 29.7 | 20.7 | 〃 | 〃 | | ⓐ 35.1 | | | | ⓐは不明 |
| 7 | 〃 | 54.9 ⓐ | 35.1 | 31.5 | 〃 | 〃 | | ⓐ 54.9 | | | | ⓐは不明 |
| 8 | 〃 | 22,5 ⓐ | 22.5 | 17.1 | 〃 | 〃 | | ⓐ 22.5 | | | | ⓐは不明 |
| 9 | a, b 17.1 | 62.1 ⓐ | 31.5 | 17.1 | 暗褐灰色 | 毛頸 | 17.1 | 26.1 | 20.7 ⓐ 62.1 | 38.7 | — | (M) ⓐは不明 |
| 10 | a, b 24.3 | 94.5 ⓐ | 45.9 | 62.1 | 黒褐灰色 | 暗毛葉下位 | 24.3 | 36.9 | 24.3 | 65.7 ⓐ 94.5 | — | (M) ⓐは(A) |
| 11 | a, b 22.5 | 74.5 ⓐ | 36.9 | 36.9 | 明黄色 | 明帯毛葉 | 22 | 31.5 | 22.5 | 56.7 ⓐ 74.5 | — | (M) ⓐは(M) |
| 12 | a, b 36.9 | 107.1 ⓐ | 51.3 | 36.9 | 明黄色 | 明帯毛葉 | 36.9 | 54.9 | 36.9 | 87.1 ⓐ 107.1 | — | (M) ⓐは(M) |
| 13 | 先代毛($G_1$)脱落後 | 121.5 | 51.3 | 36.9 | 明黄色単生毛幼毛 | 明帯毛葉 | | | | ⓐ 121.5 | | ⓐは(M) |
| 14 | 〃 | 24.3 | 24.3 | | 薄墨色単生毛青毛 | 毛柄 | | ⓐ 24.3 | | | | ⓐは不明 |
| 15 | 〃 | 31.5 | 26.1 | 17.1 | 〃 | 毛柄 | | ⓐ 31.5 | | | | ⓐは不明 |
| 16 | a 49.5 b 33.3 c 35.1 | | | | 後継毛でなく，単生の先代毛($G_1$) | 毛柄 | | | | | | |

第61表　生後1ケ月幼獣の背毛群毛包の分類（10毛群）

動物記号 K. N. 1. 背毛

| 毛の世代と発育の記号 | 3原毛包 | | | | | 副側毛包 | | | | 総毛包量 | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 | 側毛包量 | 合計毛包量 | | 合計毛量 | 毛包量 | | 毛量 | | | | |
| | no. | no. | no. | % | no. | no. | % | no. | % | no. | no. | % |
| $SG_1O$ | 7 | 20 | 27 | 90 | 27 | 28 | 66.7 | 28 | 66.7 | 55 | 55 | 76.4 |
| $SG_1Y$ | 3 | | 3 | 10 | 3 | 14 | 33.3 | 14 | 33.3 | 17 | 17 | 23.6 |
| 計 | 10 | 20 | 30 | 100 | 30 | 42 | 100 | 42 | 100 | 72 | 72 | 100 |

毛量は毛包量に等しく，1毛群の毛量の平均は7.2である

第61表続　各態生育毛量の総毛包量に対する比率

| | | |
|---|---|---|
| 毛包量 | 72 | 100 % |
| 幼毛量 | 0 | 0 〃 |
| 青毛量 | 17 | 23.6 〃 |
| 成毛量 | 55 | 76.4 〃 |
| 総毛量 | 72 | 100 〃 |

第62表　生後1ケ月幼獣の腹毛群毛包の分類（15毛群）

動物記号 K. N. 1. 腹毛

| 毛の世代と発育の記号 | 3原毛包 | | | | | 副側毛包 | | | | 総毛包量 | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 | 側毛包量 | 合計毛包量 | | 合計毛量 | 毛包量 | | 毛量 | | | | |
| | no. | no. | no. | % | no. | no. | % | no. | % | no. | no. | % |
| $SG_1O$ | 1 | 2 | 3 | | 3 | 41 | | 41 | | 44 | 44 | |
| $SG_1Y$ | | | | | | 50 | | 50 | | 50 | 50 | |
| $SG_1C$ | 3 | | 3 | | 3 | | | | | 3 | 3 | |
| | 4 | 2 | 6 | | 6 | 91 | | 91 | | 97 | 97 | |

毛量は毛包量に等しく，1毛群の毛量の平均は6.5である

第62表続　各態生育毛量の総毛包量に対する比率

| | | |
|---|---|---|
| 毛包量 | 97 | 100 % |
| 幼毛量 | 3 | |
| 青毛量 | 50 | |
| 成毛量 | 44 | |
| 総毛量 | 97 | |

動物記号 K. N. 2. 背毛　　第63表　生後2ケ月幼獣の背毛群毛包の分類（23毛群）

| 毛の世代と発育の記号 | 3 原毛包 中央毛包量 no. | % | 側毛包量 no. | % | 毛包量合計 no. | % | 毛量合計 | 副側毛包 毛包量 no. | % | 毛量 no. | % | 総毛包量 no. | 総毛量 no. | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $SG_2C$ | 1 | 4.3 | 7 | 17.4 | 8 | 11.6 | $G_2C$. 64 | $Sg_1C$. 15 | 8.9 | C. 61 | | SC. 23 | C. 125 | 34.8 |
| | | | | | | | | $Sg_1Y$. 65 | 38.5 | Y. 84 | | SY. 65 | Y. 84 | 23.4 |
| $SG_1O$ | 5 | 21.7 | | | 5 | 7.2 | $G_1O$. 61 | $Sg_1O$. 24 | 14.2 | O. 89 | | SO. 29 | O. 150 | 41.8 |
| $PG_1O.G_2C$ | 17 | 73.9 | 39 | 82.6 | 56 | 81.2 | | $PG_1O.G_2C$. 46 | 27.2 | | | PO. C. 102 | | |
| | | | | | | | | $PG_1O.G_2Y$. 19 | 11.2 | | | PO. Y. 19 | | |
| 計 | 23 | 99.9 | 46 | 100 | 69 | 100 | 125 | 169 | 100 | 234 | | 238 | 359 | 100 |

第63表 続　各態生育毛量の総毛量に対する比率

| | | |
|---|---|---|
| 3 原毛 | | |
| 毛包量 | 69 | 100 |
| 幼毛量 ($G_2.C$) | 64 | 92.8 |
| 成毛量 ($G_1.O$) | 61 | 88.4 |
| 総毛量 ($O+C$) | 125 | 181.2 |
| 副側毛 | | |
| 毛包量 | 169 | 100 |
| 2次副側毛 {$g_1$ ($Y+C$)} | 80 | 47.3 |
| 2代後継発育毛量 {$G_2$ ($Y+C$)} | 65 | 38.5(−) |
| 成毛量 ($O$) | 89 | 52.7 |
| 総毛量 ($O+Y+C$) | 234 | 138.5 |

第64表　生後2ケ月幼獣の腹毛群毛包の分類（12毛群）

動物記号 K. N. 2. 腹毛

| 3 原毛包 | | | | | | | 副側毛包 | | | | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 毛の世代と発育の記号 | 中央毛包量 | | 側毛包量 | | 毛包量合計 | | 毛量合計 | 毛の世代と発育記号 | 毛包量 | | 毛量 | 総毛量 | |
| | no. | % | no. | % | no. | % | | | no. | % | no. | no. | % |
| $SG_2C$ | | | | | 1 | | C. 5 | $Sg_1C$, | 8 | | C. 25 | C. 30 | |
| $SG_2Y$ | | | | | 2 | | Y. 11 | $Sg_1Y$, | 11 | | Y. 32 | Y. 43 | |
| $SG_1Y$ | | | | | 1 | | O. 12 | SO | 24 | | O. 62 | O. 74 | |
| $PG_1O.G_2C$ | | | | | 4 | | | $PG_1O.G_2C$, | 17 | | | | |
| $PG_1O.G_2Y$ | | | | | 8 | | | $PG_1O.G_2Y$, | 21 | | | | |
| 計 | | | | | 16 | | 28 | | 81 | | 119 | 147 | |

第64表 続　各態生育毛量の総毛量に対する比率

| | | |
|---|---|---|
| 3 原毛 | | |
| 毛包量 | 16 | 100 % |
| 幼毛量 ($G_2C$) | 5 | |
| 青毛量 ($G_2Y+G_1Y$) | 11 | |
| 成毛量 ($G_2O$) | 12 | |
| 総毛量 (O+Y+C) | 28 | |
| 副側毛 | | |
| 毛包量 | 81 | 100 |
| 2次副側毛 {$g_1$ (Y+C)} | 19 | 23.5 |
| 2代後継発育毛 {$G_2$ (Y+C)} | 38 | 46.9 |
| 成毛量 (O) | 62 | 76.5 |
| 総毛量 (O+Y+C) | 119 | 146.9 |

第65表　生後3ケ月幼獣の背毛群毛包の分類（10毛群）

動物記号 K. N. 3. 背毛

| 3 原毛包 | | | | | | | | 副側毛包 | | | | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 毛の世代と発育の記号 | 中央毛包量 | | 側毛包量 | | 毛包量合計 | | 毛量合計 | 毛の世代と発育の記号 | 毛包量 | | 毛量 | 総毛量 | |
| | no. | % | no. | % | no. | % | no. | | no. | % | no. | no. | % |
| $SG_3C$ | 2 | 20 | 1 | 5 | 3 | | 3 | $Sg_1C$ | 6 | 3.5 | 6 | C. 56 | 17.4 |
| $SG_2Y$ | | | 3 | 15 | 3 | | 3 | $Sg_1Y$ | 11 | 6.4 | 11 | Y. 74 | 23.1 |
| $SG_2O$ | 5 | 50 | 2 | 10 | 7 | | 7 | $Sg_1O$ | 55 | 32.0 | 55 | O. 191 | 59.5 |
| $PG_2O.G^3C$ | 2 | 20 | | | 2 | | 2 | $Pg_1O.g_2C$ | 43 | 25.0 | 86 | | |
| | | | | | | | | $Pg_1O.g_2Y$ | 14 | 8.1 | 28 | | |
| $PG_1O.G_2Y$ | | | 10 | 50 | 10 | | 20 | $PG_1O.G_2Y$ | 36 | 20.9 | 72 | | |
| $PG_1O.G_2O$ | 1 | 10 | 3 | 15 | 4 | | 8 | $PG_1O.G_2O$ | 6 | 3.5 | 12 | | |
| $TG_1O.G_2O.G_3C$ | | | 1 | 5 | 1 | | 3 | $TG_1O.G_2O.G_3C$ | 1 | 0.6 | 3 | | |
| 計 | 10 | 100 | 20 | 100 | 30 | | 48 | | 172 | 100 | 273 | 321 | 100 |

第65表 続　各態生育毛量の総毛量に対する比率

| | | |
|---|---|---|
| 総毛包量 | 202 | 100 % |
| 幼毛量 | 56 | 27.7 |
| 青毛量 | 74 | 36.6 |
| 成毛量 | 191 | 94.6 |
| 総毛量 | 321 | 158.9 |

第66表　生後3ケ月幼獣の腹毛群毛包の分類（7毛群）

動物記号 K. N. 2. 腹毛

| 発育記号 | 3原毛包 | | | | | 副側毛包 | | | | 総毛包量 | | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 | 側毛包量 | 毛包量合計 | | 毛量 | 毛包量 | | 毛量 | | | | | |
| | no. | no. | no. | % | 合計 | no. | % | no. | % | no. | % | no. | % |
| SC. | 1 | | 1 | | 1 | | | | | 1 | 0.6 | C. 26 | 11.0 |
| SY. | 1 | | 1 | | 1 | 2 | 1.3 | 2 | 0.9 | 3 | 1.9 | Y. 55 | 23.3 |
| SO. | 3 | 4 | 7 | | 7 | 71 | 47.3 | 71 | 31.3 | 78 | 49.1 | O. 155 | 65.7 |
| PO. C. | | | | | | 25 | 16.7 | 50 | 22.0 | 25 | 15.7 | | |
| PO. Y. | | | | | | 52 | 34.7 | 104 | 45.8 | 52 | 32.7 | | |
| | 5 | 4 | 9 | | 9 | 150 | 100 | 227 | 100 | 159 | 100 | 236 | 100 |

第66表 続　各態生育毛量の総毛包量に対する比率

| | | |
|---|---|---|
| 総毛包量 | 159 | 100 % |
| 幼毛量 (C) | 26 | 16.4 |
| 青毛量 (Y) | 55 | 34.6 |
| 成毛量 (O) | 155 | 97.5 |
| 総毛量 (O+Y+C) | 236 | 148.4 |

第67表　生後4ケ月幼獣の背毛群毛包の分類（16毛群）

動物記号 K. N. 4. 背毛

| 毛の世代と発育記号の組合 | 3原毛包 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 | | 側毛包量 | | 毛包量合計 | | 毛量合計 | |
| | no. | % | no. | % | no. | % | no. | % |
| $SG_3C$ | 3 | 19 | | | 3 | 6.3 | C 7 | 11.1 |
| $SG_2O$ | | | 12 | 37.5 | 25 | 52.1 | Y 3 | 4.6 |
| $PG_2O.G_3C$ | 13 | 81 | 4 | 12.5 | 4 | 8.3 | O 58 | 85.3 |
| $PG_2O.G_3Y$ | | | 3 | 9.4 | 3 | 6.3 | | |
| $PG_1O.G_2O$ | | | 13 | 40.6 | 13 | 27.1 | | |
| | 16 | 100 | 32 | 100 | 48 | 100.1 | 68 | 100 |

註. 副側毛量は不備の為め第68表に従う.

第68表　生後4ケ月幼獣の背毛群毛包の分類（5毛群）

動物記号　K. N. 4.　背毛

| 3 原毛包 | | | | | 副側毛包 | | | | | | 総毛量 | | 1毛群平均毛量 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 毛の発育の世代と記号 | 中央毛包量 no. | 側毛包量 no. | 毛包合計量 | 毛合計量 | 毛記号の発育 | 毛包量 no. | 毛の発育の世代と記号組合 | 毛包量 no. | 毛包量 % | 毛量 no. | no. | % | |
| $SG_3C$ | 2 | | 2 | 2 | SC | 4 | $Sg_1C$ | 4 | 2.5 | 4 | C. 39 | 10.6 | |
| | | | | | SY | 1 | $Sg_1Y$ | 1 | 0.6 | 1 | Y. 42 | 19.0 | |
| $SG_2O$ | 3 | 2 | 5 | 5 | SO | 65 | $Sg_1O$ | 65 | 40.9 | 65 | O. 193 | 70.4 | |
| $PG_2O.G_3C$ | | 4 | 4 | 8 | PO. C. | 29 | $Pg_1O.g_2(Y+C)$ | 57 | 35.8 | 114 | | | |
| $PG_2O.G^3Y$ | | 1 | 1 | 2 | PO. Y. | 37 | $PG_2O.G_3(Y+C)$ | 9 | 5.7 | 18 | | | |
| $PG_1O.G_2O$ | | 3 | 3 | 6 | PO. O. | 20 | $PG_1O, G_2O$ | 20 | 12.6 | 40 | | | |
| | | | | | TO. O. C. | 3 | $TG_1O, G_2O, G_3C$ | 3 | 1.9 | 9 | | | |
| 計 | 5 | 10 | 15 | 23 | | 159 | | 159 | 100 | 251 | 274 | 100 | 55 |

総毛包量　174　100%　　総毛量　274　157%

第69表　生後4ケ月の幼獣の腹毛群毛包の分類（10毛群）

動物記号　K. N. 4.　腹毛

| 毛の発育記号 | 3 原毛包 | | | | 副側毛包 | | | 総毛包量 | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 no. | 側毛包量 no. | 毛包量合計 no. | 毛量合計 | 毛包量 | 毛量 no. | % | | no. | % |
| SC | 1 | | 1 | 1 | | C. 14 | 4.8 | | C. 15 | 5 |
| SY | | | | | | Y. 43 | 14.7 | | Y. 43 | 14.3 |
| SO | 4 | 2 | 6 | 6 | | | | | | |
| PO. C | | | | | | O. 236 | 80.5 | | O. 242 | 80.7 |
| PO. Y | | | | | | | | | | |
| PO. O | | | | | | | | | | |
| | 5 | 2 | 7 | 7 | | 293 | 100 | | 300 | 100 |

第70表　生後8ケ月幼獣の背毛群毛包の分類（5毛群）

動物記号　K. N. V—8.　背毛

| 毛の発育記号 | 3 原毛包 | | | | | 副側毛包 | | | | 総毛包量 | | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 no. | 側毛包量 no. | 毛包量合計 no. | % | 毛量 no. | 毛包量 no. | % | 毛量 no. | % | no. | % | no. | % |
| SC | 1 | 1 | 2 | | C. 5 | 8 | 5.8 | C. 64 | | 10 | 6.8 | C. 69 | 28.0 |
| SY | | 1 | 1 | | Y. 1 | 17 | 12.2 | Y. 45 | | 18 | 12.2 | Y. 46 | 18.7 |
| SO | 3 | | 3 | | O. 6 | 26 | 18.7 | O. 125 | | 29 | 19.6 | O. 131 | 53.3 |
| PO. C | 1 | 2 | 3 | | | 49 | 35.3 | | | 52 | 35.1 | | |
| PO. Y | | | | | | 28 | 20.1 | | | 28 | 18.9 | | |
| PO. O | | | | | | 4 | 2.9 | | | 4 | 2.7 | | |
| TO. O. C | | | | | | 7 | 5.0 | | | 7 | 4.7 | | |
| | 5 | 4 | 9 | | 12 | 139 | 100 | 234 | | 148 | 100 | 246 | 100 |

第70表続　各態生育毛量の総毛包量の総毛包量に対する比率

| | | |
|---|---|---|
| 総毛包量 | 148 | 100 % |
| 幼毛量 | 69 | 46.6〃 |
| 青毛量 | 46 | 31.1〃 |
| 成毛量 | 131 | 88.5〃 |
| 総毛量 | 246 | 166.2〃 |

第71表　生後8ケ月幼獣の腹毛群毛包の分類（5毛群）

動物記号　K. N. V—8. 腹毛

| 毛の発育記号 | 3原毛包 中央毛包量 no. | 3原毛包 側毛包量 no. | 3原毛包 毛包量合計 no. | 3原毛包 毛量合計 no. | 副側毛包 毛包量 no. | 副側毛包 毛包量 % | 副側毛包 毛量 no. | 副側毛包 毛量 % | 総毛包量 no. | 総毛量 no. | 総毛量 % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SC | 1 | 2 | 3 | 3 | 4 | 2.5 | 4 | | 7 | C. 36 | 12.7 |
| SY | 1 | 4 | 5 | 5 | 45 | 28.0 | 45 | | 50 | Y. 116 | 40.8 |
| SO | 3 | | 3 | 3 | 16 | 9.9 | 16 | | 19 | O. 132 | 46.5 |
| PO. C | | 2 | 2 | 4 | 15 | 9.3 | 30 | | 17 | | |
| PO. Y | | | | | 66 | 41.0 | 132 | | 66 | | |
| PO. O | | | | | 3 | 1.9 | 6 | | 3 | | |
| TO. O. C | | | | | 12 | 7.5 | 36 | | 12 | | |
| | 5 | 8 | 13 | 15 | 161 | 100.1 | 269 | | 174 | 284 | 100 |

第71表続　各態生育毛量の総毛包量の総毛包量に対する比率

| | | |
|---|---|---|
| 総毛包量 | 174 | 100 % |
| 幼毛量 | 36 | 20.7〃 |
| 青毛量 | 116 | 66.7〃 |
| 成毛量 | 132 | 75.9〃 |
| 総毛量 | 284 | 163.2〃 |

R切片による2ケ月幼獣の毛群組成の観察

2ケ月幼獣のR切片の毛束の解体調査によれば，単生の青毛は先代の棍状毛の落毛後の対生毛から出来た単生毛ではなくて新に副生した2次副側毛（post sublateral hair）である．これら青毛の多くは皮脂腺位の断片では，後方に位置を占めていて，先代棍状毛の落毛の痕跡を留めていない．又資料採取期は12月15日で寒冷季であるので下毛の落毛は稀れである，ことなどにより立証される．2次副側毛は毛世代記号は $g_1$ で，青毛の発育記号は $g_1Y$ である．又中央毛，側毛等の上毛は先代毛（生毛 $G_1$）と後継毛（$G_2$）の比較では明らかに後継毛は飛躍的に形態の増大を示して，Dry の所謂 step-up（累世加大？）が認められる．下毛では換毛により，step-up，step-down 又は旧態維持の3つの状態を保つている．

R切片による3ケ月幼獣の毛群組成の観察

検鏡によれば背毛群の毛束の平均数は凡そ7.3で，3原毛の毛包数を差引けば残りの4.3は生毛の副側毛包数と同数であると考えられる．それで10毛群には43の副側毛包がある筈である．1方10背毛群の調査によれば副側毛は $PG_1O.G_2Y.$ $PG_1O.G_2O.$ $TG_1O.G_2O.G_3C.$

の毛束（毛包数に同じ）も43で過然に一致している．棍状毛2個を含む毛束7は次のような方法で表示出来る．即ちそれらの内ただ1毛束は2個の棍状毛と1個の幼毛を含む毛束で毛世代記号と発生記号の組合せで $TG_1O.\ G_2O.\ G_3C$ 同様に残りの6毛束は $PG_1O,\ G_2O,$ である．次に成毛と青毛の対毛束（PO, $Y_1$）の50毛束は次のように分割される．即ち43−7=36毛束は生毛の副側毛包から生じて $PG_1O.\ G_2Y$ の発生記号で表わされる．したがつて50−36=14毛束は2次副側毛包から発生したもので $Pg_1O,\ g_2Y$ の発生記号で表わされる．単生の棍状毛の凡ては2次副側毛 $g_1O$ で生毛の副側毛 $G_1O$ ではない．なんとなれば同毛毛包に後継毛の痕跡はない．同様に単生の SY, SC は2次副側毛 $Sg_1Y$, $Sg_1C$ である．

R切片による4ケ月幼獣の毛群組成の観察．

中央毛は凡て独立毛包の単生棍状毛と幼毛である．棍状毛はいづれも毛根部の横断面で中心部に色素顆粒が点在している．幼毛は毛葉の横断面を表わしている．棍状毛は成長を停止して次代の後継毛が下部から生育して来るのを待ち受け，幼毛は先代の棍状毛が落毛してまだその痕跡を留めている．以上の観察よりこの幼毛の先代毛は前述の単生棍状毛と同期のものに相違いない．さらにこの棍状毛の毛根の大さと同大の毛根を持つている全毛を採毛調査表から索めてその葉部とこれらの幼毛の断面と対照すると明らかな差異がないので，これのみでは所謂 step-up, とか step-down とかの判断はし難い．生後2ケ月の幼獣と3ケ月幼獣との調査の結果より生毛（$G_1$）とその後継毛（$G_2$）の間には極端な type step-up の変化があるのと，毛の換毛期間の推定からこれらの継続2代は $G_2$ と $G_3$ と認められる．又側毛では明らかに type step-up を表わしている．成毛の対生 $PG_1O.\ G_2O$ 或は成毛2個幼毛1個の3連生 $TG_1O.\ G_2O.\ G_3C$ が観察されることからも中央毛の考察は正しい（写142図）．次に3原毛をのぞいた副側毛であるが，生後4ケ月は2月に当り酷寒期で棍状毛の落毛が遅延していて成毛の対生 PO. O は特に多数である．これらは大型下毛が多く，根幅は郭大の傾向があり，$PG_1O.\ G_2O$ と認められる．なお多数の SO. SY. SC（単生毛）これらは先代毛が落毛した後の痕跡がなく，皮脂腺位で偽毛束解体により SO, SY, SC の順序に配置されており，2次副側毛の定形的な発生順位を表わしている（写140, 143図）．

又生毛の毛包数の調査によると1毛群の平均は9.4である．これより3原毛を差引き 6.4 が副側毛分の毛包となり，5毛群では 6.4×5=32 の副側毛がある訳けである．しかるに5毛群で明らかに生毛包から発生したと見られるものは $PG_1O.\ G_2O$=20, $TG_1O.\ G_2O.\ G_3C$=3 との23毛包であるので，32−23=9 の毛包が過剰となる．検鏡によれば S. O. は先代毛の落毛の痕跡はないが，PO. C, PO. Y において落毛の痕跡が処々に認められるので，3連生毛の先先代（$G_1O$）の棍状毛の落毛後の対生毛と考察してこれを $PG_2O.\ G_3C$, $PG_2O,$ $G_3Y$ であるといえよう．従つて過剰の対生毛包の9は $G_1O$ を失つた $PG_2O.\ G_3Y$ 或は $PG_2O.\ G_3C$ といえよう．以上の理由により第68表のような結果が生じる．

R切片による8ケ月幼獣の毛群組成の考察．

（総幼毛64）−（対生幼毛56）=8 幼毛の単生幼毛があるが，この際新らたに下毛が副生することと，対生の成毛の落毛と2つの場合が考えられる．小型下毛では後継毛が幼毛の内に成毛の落毛することはほとんどない．とくに資料の採取は11月20日であるので成毛の残留の確率は高い．検鏡の結果明らかに新副側毛の発生の事実があるので生後8ケ月にして未だに新毛包が増加していることを認めえた．

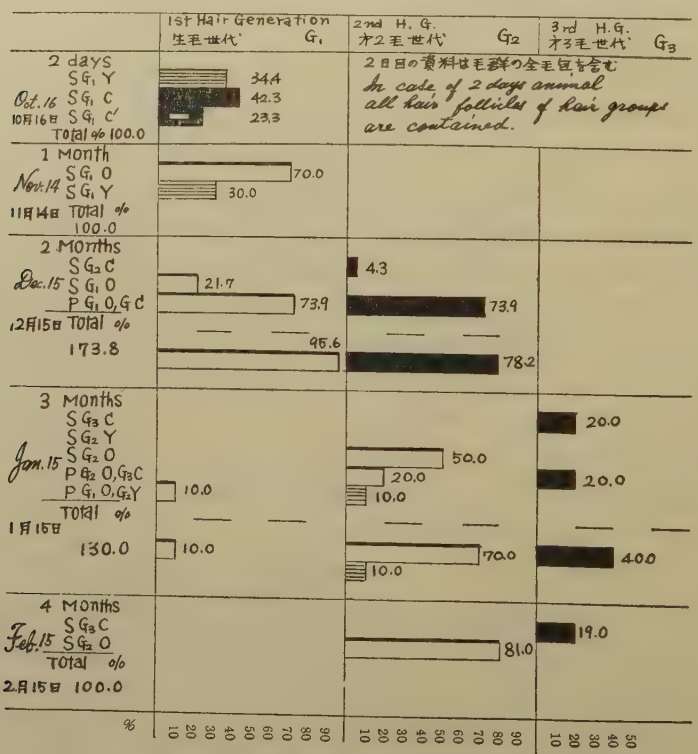
(総青毛45)−(対生青毛28)=17青毛の単生青毛があるが，これは幼毛と同様新しく副生したものか，対生の成毛が落毛した後かであるが，11月以後の生態を表わしているので最早成毛の落毛は稀れであることから，これ等は後継毛と新生毛の両者を含んでいることは明らかである．

第37図 毛世代と各毛相との関係

The Relation between Hair Generation and Hair developping Phase

生後 1, 2, 3, 4ケ月の被毛における中央毛の発生過程各相の毛量比較図
(毛母基を 100% とする)

Comparative chart of ratio of the central original hairs no. of each developping phase to their total matrices' no. from the pelages of 1, 2, 3 and 4 monthos animals.



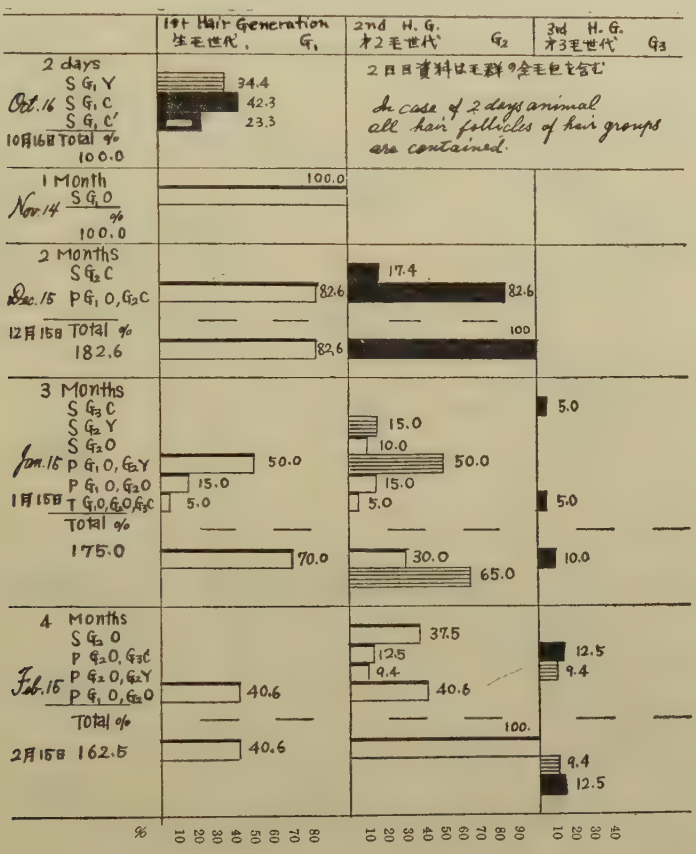
第38図　毛世代と各毛相との関係

The Relation between Hair Generation and Hair developping Phase

生後 1, 2, 3, 4ケ月の被毛における側毛の発生過程各相の毛量比較図

（毛母基を 100％とする）

Comparative chart of ratio of the lateral original hairs' no. of each developping phase to their total matrices' no. from the pelages of 1, 2, 3, and 4 months animals.

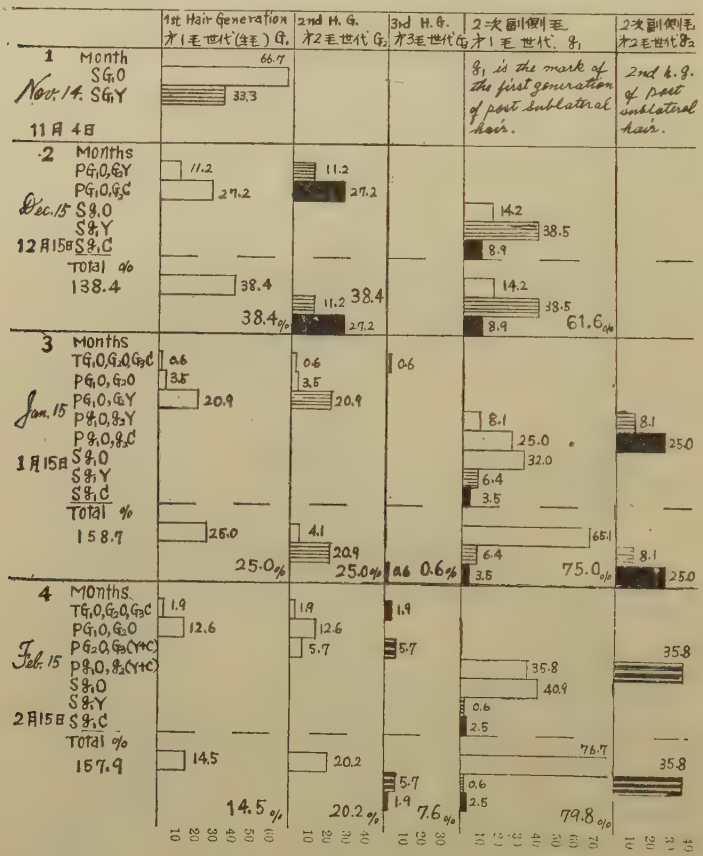


第39図 毛世代と各毛相との関係

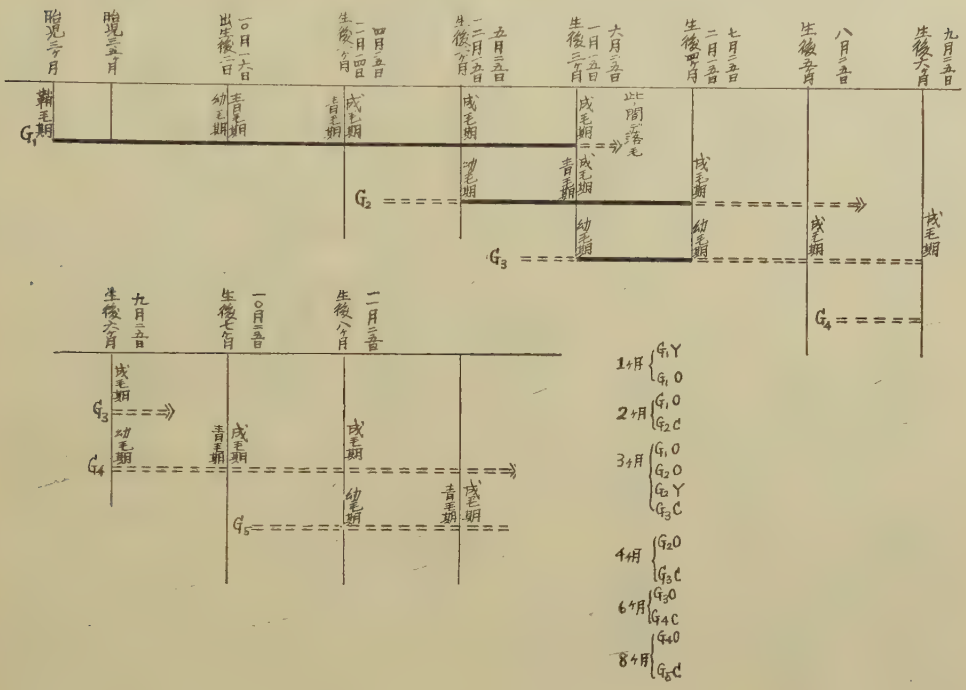
The Relation between Hair Generation and Hair developping Phase (Ⅱ)

生後 1, 2, 3, 4ケ月幼獣の被毛における副側毛の毛母基に対する発生過程各相の毛量比較図（毛母基を100％とする）

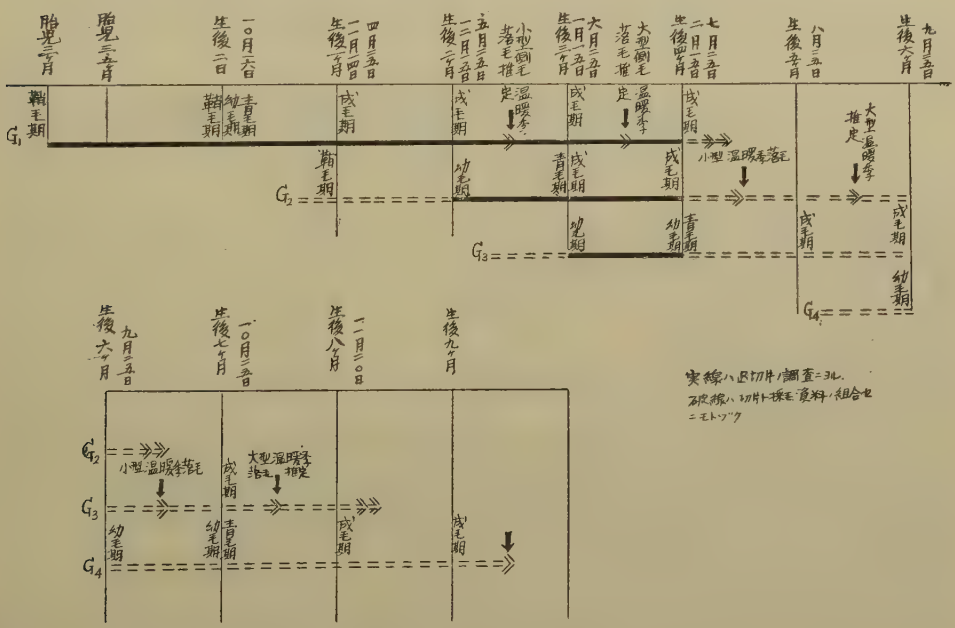
Comparative chart of ratio of the sublateral hairs' no. of each developping phase to their total matrices' no. from the pelages of 1, 2, 3 and 4 months animals.



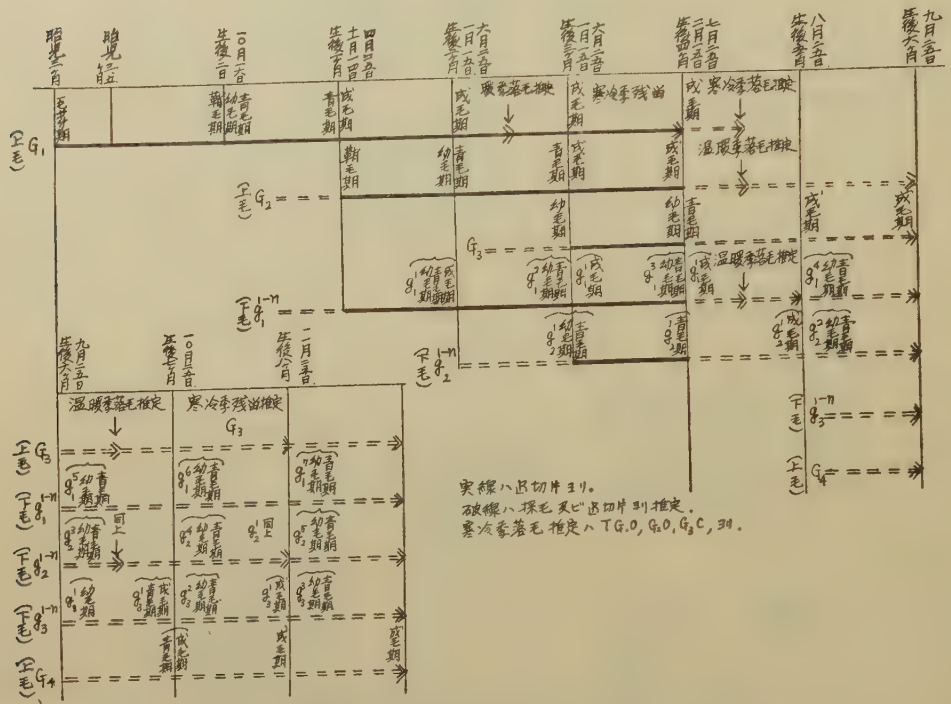
第40図　中央毛の発生と換毛 (Development and Replacement of Central Hairs)




第41図　側毛の発生と換毛 (Development and Replacement of Lateral Hairs)



第42図 小型上毛，分岐毛下毛の発生と換毛（Development and Replacement of small type Over-hair, Cross-hair and Under-hair.）




上図表により略次のような各種被毛について毛世代の推移が考察される．

第 72 表

| | | (毛芽より成長停止の棍状毛までの期間) 月数 | (温暖季の毛芽より落毛までの期間) 月数 | (寒冷季の毛芽より落毛までの期間) 月数 |
|---|---|---|---|---|
| 第一上毛 | $G_1$ | 3 | 5.5 | 5.5 |
| | $G_2$—n | 2—2.5 | 4.5—5.5 | 4.5—5.0 |
| 第二上毛 | $G_1$ | 3 | 4.5 | 6.5 |
| | $G_2$—n | 2—2.5 | 3.5—4.0 | 5.5—6.0 |
| 小型第二上毛，分岐毛及び下毛 | $G_1$又は$g_1$ | 2.5 | 4.0 | 6.0 |
| | $G_2$—n又は$g_2$—n | 2.0 | 3.5 | 5.5 |

上記のように毛原基の陥入より始まる生毛世代（第1毛世代 $G_1$，中央毛，側毛，副側毛を含む）及び2次副側毛第1毛世代（$g_1$）の週期は第2毛世代以後 $G_2$-n, $g_2$-nよりやや長期間を経過するように考察される．要するに Nutria では生後約8ケ月→12ケ月において成獣の正常の被毛に換毛することになる．

6. 換毛波 (molt wave)

Dry (1926) は mouse (Mus muscus) で又 Collins[9] (1923) は mouse (Peromyscus maniculatus gambeli) で生毛の発生から漸次換毛を研究して，その毛包の発生の順序は概して咽喉部，胸部，腹部より始まり腹側をのぼり，肩部，後頭部を廻つて下向して背部に向

つて押し寄せ臀端に下つて全体の生毛（$G_1$）の発生を完成するに約1ケ月経過していることを述べている．その後継毛の毛世代にも（$G_2 \rightarrow G_3 \rightarrow G_4$）略同期間に同様な方向を辿つて行われている．この規則的に定期的に進む換毛を換毛波（molt wave）と呼んでいる．Nutria の生毛は3ケ月胎児で調査の結果のように頭部，背部の発生が早く，続いて腹部，胸部に進むもののようである．出生後の資料は背部の中央と腹部の中央のみにすぎないので明瞭でないが，生毛群の内大型の上毛では気温の寒暖の影響も少く規則的に換毛するので成毛が一掃されて後継毛の幼毛（毛長の半ば，毛頸まで成長）のみで被われることもあるので換毛波の経過が推測されるが側毛以下の中小上毛は成毛の滞留期が次の毛世代の青毛期を経て寒冷季では成毛期まで，更にその後継幼毛を含む3連生毛（TGnO. $Gn_{+1}$O. $Gn_{+2}$C）までも延長するので部分的に同じ成長期の被毛が揃うことは外観的には観察が困難で $G_2$ の換毛期よりすでに明白でない．とくに副側毛（下毛）の後継毛と2次副側毛（下毛）のように漸次間断なく発生する場合は同じ個所に種々複雑に発育過程の毛包が観察されて規則的な換毛経過は認められない．生後2次的に下毛が増加して偽毛束を形成する動物では $G_1$ の他は換毛波は考えられない．mouse のように単生毛包のみで構成される真毛束の単純毛群の場合のみ換毛波が認められるようである．

7. 毛量の変遷

Wilcox[43]（1950）の例のように Chinchilla の生毛は単生毛であるが，成獣は中央上毛を挟んで両側毛は分岐して2個の真毛束となり，1毛群は最高75毛に増加する．Dry[11]（1926）の例のように mouse は生後約1ケ月で単生の生毛を完成するが，その後約3ケ月で成獣となり，生後4ケ月では毛世代の進行によつて毛束は3～4ケ月（12—17 weeks）に2次毛包の増殖がある（secondary follicles）．

このように生後動物の発育に伴つて体表面は拡張して，毛群の密度は激減するが2次下毛の分岐，分根によつて毛量の調節が行われると言う．Nutria の場合も既に述べたように，2次副側毛（下毛）が胚芽層から陥入して発生し生後略12ケ月頃まで漸増している．これを次のような方法で調査観察して図表のような結果を得た（第73表，第44，45図）．

(1) 皮面に水平な切片の資料H切片の作製可能の場合は直接検鏡の視野の面積と1 $cm^2$ との比率で算定した．

(2) 毛包に直角な切片の資料R切片，とH切片の両者が作製可能の場合は両者を検鏡して同視野で求めた両者の数の比例より次のように毛包の傾斜角が算定される．但しR切片のみでは皮面の毛包数は算出出来ないので，他の個体の資料で得た毛包の傾斜角を使用して毛量を推計した．

(3) 又は生皮を脱毛して鞣革としてその皮面の毛痕跡によつて毛群数を算定する方法を用いた．

(4) 毛包の傾斜角計算法 Chase[10]（1954）によれば毛芽は始め表皮に直角に下降発育しているが mouse では生後3日で背部被毛の毛包は通常 30° の角度を保つて傾斜を示して来るとしている．筆者の場合 Nutria も同じように略一定の角度を保つているようである（但しホルマリン漬固定の資料による）．

H切片とR切片との検鏡視野における毛量或は毛群数を知つて，毛の傾斜角を求める方法を考案した．顕微鏡の視野の半径をrとする．R切片の視野内の毛包数又は毛群数はH切片の表皮面内では作図（第43図）のように短径を2r，長径を2r′とする楕円形の面積内に散在する毛包数又は毛群数に等しい．今H切片の視野内の毛包数をA，R切片の視野内の毛

包数をBとせば次のようになる。$\frac{A}{B}=\frac{\text{円の面積 S}}{\text{楕円の面積 S'}}=\frac{r^2\pi}{r'r\pi}=\frac{r}{r'}$未知の角を $\theta°$ とせば $\mathrm{Sin}\theta°=\frac{r}{r'}=\frac{A}{B}$である。調査の経過並びにその成績2例は次のようである.

（第1例）　**3ケ月胎児の毛群の観察**

背部被毛の密度（背部単位面積内（Per sq. cm²）の幼毛，鞘毛，球栓毛及び毛原基の数）検鏡倍率100倍の視野は $S=r^2\times3.14$, $R=1.34$ mm で $S=\left(\frac{1.34}{2}\right)^2\times3.14=1.409546$ mm² である。

H切片の実測によればこの視野の毛芽，毛原基（germ of hair and anlage of follicle）を混えた将来3原毛群（trio orginal hairs' group）となるものは平均68群で，1 cm² 内には $68\times\frac{100}{1.409546}=4,824$ 毛群である．切片の標本によると，これら毛包群は中央に大型の幼毛を含み両後側に小型の幼毛が接触して3原毛を配してその他の副側毛は未だ毛芽で，全15毛包を含んで1毛群を形成する．最大生毛群の場合．中央毛は鞘毛，両側毛は球栓毛で，その他は毛原基の副側毛で形成されている．全7毛包の生毛群の場合．中央毛が球栓毛で両側毛が未だ毛原基の3生毛群の場合及びそれ等の中間型の生毛群である．幼毛，鞘毛，球栓毛は黒褐色の色素を含んだオタマヂヤクシ様である．

染色無処理の透明標本（皮膚 whole skin）を表皮面から透視観察の場合は，生毛群の組織の観察は不能であるが濃い黒褐色の色素を含むために幼毛，鞘毛，球栓毛の存在は明らかである．黒褐色の鞘毛は表皮面に芒先を現わす寸前のもの又は既に毛球を皮内深く潜入させ芒先（tip of awn）を毛管（hair canal）外の表皮面に現わして幼毛の列に加わらんとしているもの等で，その平均の観察数は検鏡の視野に58.8である．1 cm² 内では $58.8\times\frac{100}{1.409546}=4,171$ の球栓毛，鞘毛，幼毛が内在することになる．これは上述の毛群数にも充たない数である．3原毛群が完全に揃つているとせば，1 cm² 内の全3原毛数は14,463となるので約28%が球栓毛，鞘毛，幼毛で72%は毛原基である．

又各生毛群（Lanugo hair group）の平均生毛包数を7とせば，1 cm² 内の全生毛数は33,747となるので約12%が球栓毛，鞘毛，幼毛で染色無処理で視野に浮び，88%は未だ毛原基でヘマトキシリンに染色可能である．3ケ月の胎児では頭，背，腹，胸の各部位に毛原基の陥入が見られるが（写20, 21図），頭部，背部は既に3原毛が幼毛，鞘毛期で芒先が毛管から表皮面に現われたもの，正に突き破つて現われんとするもの等である．腹部は球栓期でいづれも黒褐色の毛芒部でオタマジヤクシのように見えるが，胸部は毛栓期，毛原基期でヘマトキシリンに染め着いてはじめて発見される．腹部，胸部では球栓期の球毛は稀れである．

即ち3ケ月胎児では3原毛群は幼毛として完全に揃つていないが，前述のように生毛の毛原基は略完全に備わつている．3ケ月胎児では肉眼的に外貌は頭部，背部が薄墨色の裸形である（写3図）.

（第2例）　**成獣の被毛密度の観察**

動物記号N—3，生体量5.4瓩，12ケ月，♀，

資料と調査方法，明礬鞣皮革とした，硬紙に0.5 cm² の窓を作り，これを皮面にあてて解剖顕微鏡で検鏡した.

(イ) 背部皮革面 1 cm² 内の毛群数

皮面 0.25 cm² の毛群数平均 32, 33, 39, 36, 37, 34, 35, 30, 33, 34の10回検鏡合計 343, 平均, 34, 3, よつて 1 cm² 内の毛群数 34.3×4=137.2

(ロ) 背部皮革面 1 cm² 内の毛量

(毛群平均毛量)×(1 cm² 内毛群数)=84.8×137.2=11, 634.56≒11, 634

(ハ) 背部 1 cm² 内の毛包量

$$11,634.56 \times \frac{227}{451} = 5,855.97 \fallingdotseq 5,856$$

註 $\frac{\text{総毛包量}}{\text{毛群の総毛量}} = \frac{227}{451}$, これは第83表にある.

(ニ) 被毛の傾斜角

ℝ切片の100倍検鏡視野内の毛群は 4, 6, 5, 5, 4, 6, 5, 5, 5, 5, の10回検鏡, 平均5毛群である.

従つてℝ切片の 1 cm² 内の毛群数は

$$\text{視野の平均毛群数} \times \frac{1\ \text{cm}^2}{\text{視野の面積}} = 5 \times \frac{100}{1.409546} = 355$$

$$\sin\theta^\circ = \frac{\mathbb{H}\text{切片内の毛群数}}{\mathbb{R}\text{切片内の毛群数}} = \frac{137}{355} = 0.38591$$

傾斜角 $\theta^\circ = 22^\circ 40' - 22^\circ 50'$

(ホ) 腹部皮革面 1 cm² 内の毛群数

腹部皮革面 0.25 cm² 内の毛群数は検鏡10回で 38, 33, 33, 40, 44, 30, 32, 38, 32, 35, 合計355, 35.5×16=568, 1 cm² 内の毛群数は568である.

(ヘ) 腹部 1 毛群の平均毛量

検鏡10毛群では 29, 43, 34, 39, 29, 32, 36, 53, 21, 32, 合計348平均34.8

(ト) 皮面 1 cm² 内の毛量

34.8×568=19, 766, 4≒19, 766

(チ) 毛包の傾斜角

ℝ切片内の平均毛群数, 10回検鏡で 16, 17, 16, 18, 20, 18, 17, 21, 18, 16合計177, 平均17.7

ℝ切片 1 cm² 内の平均毛群数 $17.7 \times \frac{100}{1,409546} = 1256$

$\sin\theta^\circ = \frac{568}{1256} = 0.4522$, 傾斜角 $\theta^\circ = 26^\circ 50' - 27^\circ$

第73表 幼獣並に成獣の毛叢の密度
Density per sq cm of juvenal and adult animal skins.

背部中央 Mid-dorsum

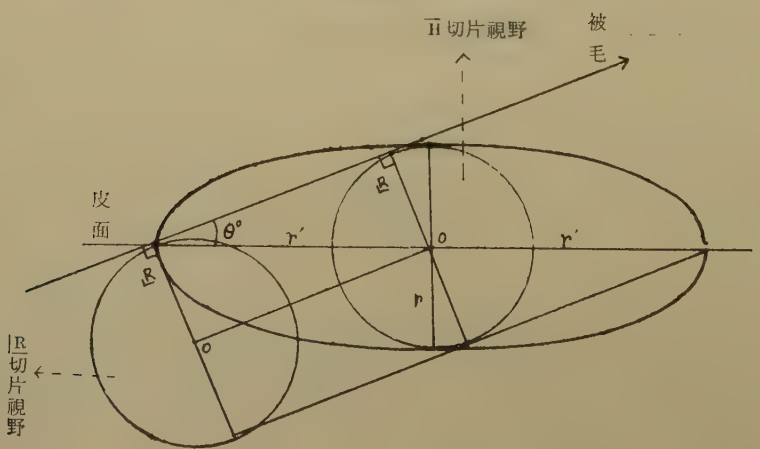
| 動物記号と年齢 No. and age of animal | | 毛群数 No. of hair group | % | 毛包数(毛母基数) No. of follicles (matrices) | % | 毛量 Sum of hairs | % | 青色と成毛 Young and club hairs 毛量 | % | 毛群の総毛量に対する比率 % |
|---|---|---|---|---|---|---|---|---|---|---|
| KN. 03 | 胎児3ケ月 | 4, 824 | 35.5 | — | — | 4, 171 | 3.4 | 0 | | 0 |
| KN. 0 | 生後2日 ♂ | 3, 775 | 27.8 | 21, 179 | 23.9 | 21, 179 | 17.0 | 7, 282 | 8.2 | 34.4 |
| KN. 1 | 生後1ケ月♂ | 3, 097 | 22.8 | 18, 336 | 20.7 | 18, 336 | 14.8 | 18, 336 | 20.7 | 100.0 |
| KN. 2 | 生後2ケ月♂ | 653 | 4.8 | 14, 450 | 16.3 | 21, 797 | 17.5 | 14, 211 | 16.0 | 65.2 |
| KN. 3 | 生後3ケ月♂ | 429 | 3.2 | 10, 172 | 11.5 | 16, 164 | 13.0 | 13, 352 | 15.1 | 82.6 |
| KN. 4 | 生後4ケ月♂ | 274 | 2.0 | 7, 697 | 8.7 | 12, 121 | 9.8 | 10, 836 | 12.2 | 89.4 |
| KNV—8 | 生後8ケ月♀ | 259 | 2.0 | 6, 112 | 6.9 | 10, 159 | 8.2 | ※7, 314 | 8.3 | 72.0 |
| N—3 | 生後12ケ月♀ | 137 | 1.0 | 6, 454 | 7.3 | 11, 634 | 9.4 | 10, 156 | 11.5 | 87.3 |
| N—1 | 生後32ケ月♀ | 131 | 1.0 | 4, 358 | 4.9 | 8, 659 | 7.0 | 7, 074 | 8.0 | 81.7 |
| | 計 | 13, 579 | 100.1 | 88, 758 | 100.2 | 124, 220 | 100.1 | 88, 561 | 100 | |

腹部中央 Mid-abdormen

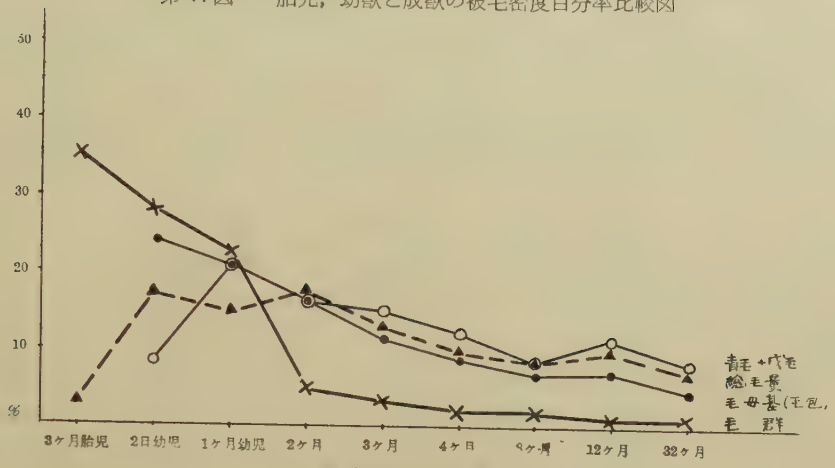
| 動物記号と年齢 No. and age of animal | | 毛群数 No. of hair group | % | 毛包数(毛母基数) No. of follicles (matrices) | % | 毛量 Sum of hairs | % | 青毛と成毛 Young and club hairs 毛量 | % | 毛群の総毛量に対する比率 % |
|---|---|---|---|---|---|---|---|---|---|---|
| KN. 03 | 胎児3ケ月 | — | | — | | — | | — | | — |
| KN. 0 | 生後2日 ♂ | — | | — | | — | | — | | — |
| KN. 1 | 生後1ケ月♂ | — | | — | | — | | — | | — |
| KN. 2 | 生後2ケ月♂ | — | | — | | — | | — | | — |
| KN. 3 | 生後3ケ月♂ | — | | — | | — | | — | | — |
| KN. 4 | 生後4ケ月♂ | 876 | | | | 19, 214 | | 18, 253 | | 95 |
| KNV—8 | 生後8ケ月♀ | — | | — | | — | | | | — |
| N—3 | 生後12ケ月♀ | 568 | | — | | 19, 766 | | | | — |
| N—1 | 生後32ケ月♀ | 569 | | — | | 19, 537 | | | | — |
| | 計 | | | | | | | | | |

註, ※は毛包の仮想傾斜角20°～30°の平均から算出した.

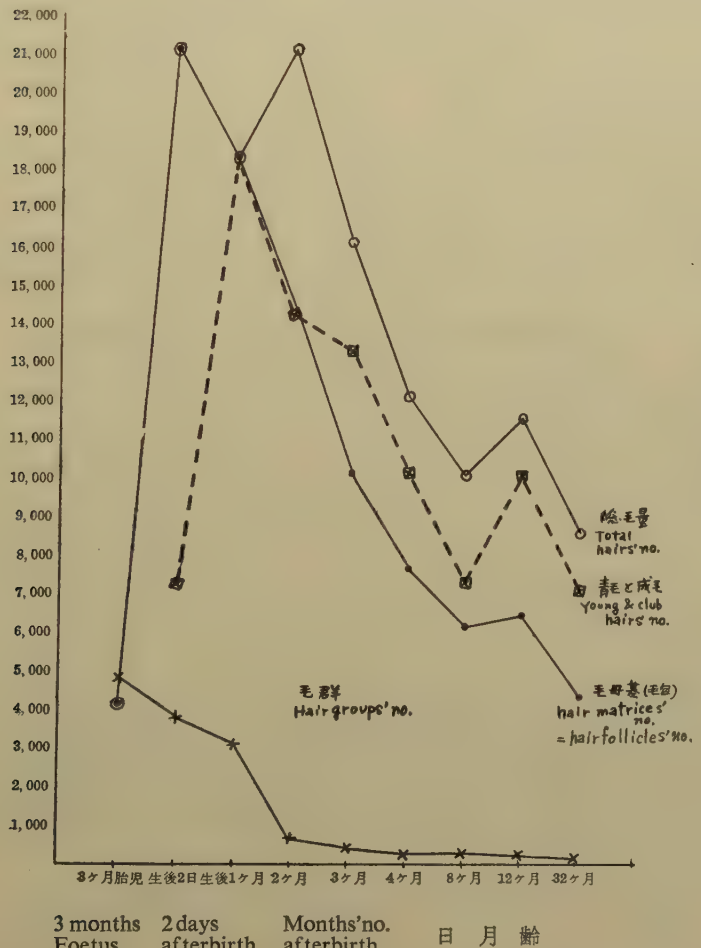
第 43 図



第44図　胎児，幼獣と成獣の被毛密度百分率比較図




第45図　胎児，幼獣，成獣の中背部 1 cm² 内の毛群，毛包，被毛量比較



図表によれば毛群数の変化は体表面の拡張即ち生体の発育成長を示している．これより生後8ケ月，12ケ月の間に Nutria の成獣完成が認められる．生後1ケ月幼獣までは毛群数は漸減しているが1ケ月から2ケ月にかけて激減している．毛包数は1－2ケ月に毛群の激減にかかわらず漸減を示しているのは，この間に最も多くの2次下毛包の増殖をみせていることから判明する（この全資料は全員が寒冷季で落毛が僅少で毛量は多く，更に条件は均等である）．

生後3ケ月幼獣の頃までは成獣の2倍量の被毛があり，幼獣期の体温保護の役を果しているようであ．るそれより漸減して成獣に到つて毛量は均衡を保つている．

更に腹部被毛は幼獣期の観察成績はないが，成獣期に入つてなお背部の略々2倍量である．

水中游泳中の保温効果は明らかである．とくにこの時期の下毛は成獣よりも繊細であるので（第31，32，33，34図），密度の高いことと相俟つて，Nutria の生涯の中最も繊美な毛皮を提供してくれるが，遺憾ながら鞣製後この毛皮で最も珍重される部分の腹面の皮革が未熟のため脆弱である．なお上坂等[41](1950) は在来白色兎及びアンゴラ兎の冬季の毛皮で研究の結果を次のように述べている．

第 74 表

| 平均 | 毛群数 ($1cm^2$ 中) | 毛束数 ($1cm^2$ 中) | 1毛群内の被毛数 | 1毛群内の毛束数 |
|---|---|---|---|---|
| 在来白色兎（冬毛） | 446 | 1,844 | 35.3 | 4.2 |
| アンゴラ兎（冬毛） | 462 | 2,084 | 36.3 | 4.1 |

これにより $1\,cm^2$ 当りの在来白色兎の被毛量は，毛群内の被毛量×$1\,cm^2$ 内の毛群数＝35.3×446＝15,743.8で，アンゴラ兎は36.3×462＝16,770.6のようである．これらと Nutria の被毛を比較するに背部毛量は兎毛皮に比して下位にあるが，腹部はやや密度が高い．

### Ⅶ. 成獣被毛の体重別（年令別）及び性別による差異

一般に動物の年齢別，性別による被毛の繊度の変化があると言われているが，過去においてこれを明確な数字で表現した文献はないようである．常識的には性別では牡の被毛が，年齢的には年長者の被毛が粗大なことになつている．筆者は Nutria で年齢別，性別により被毛の変化を検討することにした．既に月齢より幼獣の被毛の変化については「生毛より成獣の被毛まで」で解き明かしたのであるが，これを12ケ月以上の成獣について次のように調査した．即ちこの動物資料は（1954年12月→1955年2月の3ケ月の寒冷季）同一寒冷季に採集が行なわれた．

Nutria 飼育者の言によればいづれも1ケ年以上の成獣で，とくに7.5瓩前後の動物は2ケ年→3ケ年飼育の老獣とのことであるが，ニュートリヤ組合に登録中のもので12ケ月以上と確認されたものから体重3.5瓩（約1貫），5.5瓩（約1.5貫），7.5瓩（約2貫）の3区に分つて，各区を牝牡5頭宛，総数30頭を採り，背毛を採集して下毛＋分岐下毛（under hair＋cross under hair）の柄幅（diameter of hair stalk）の平均値を求めて各々均等性（homogeneity）について検討した。

第75表　1954年12月～5年2月，寒冷季3ヶ月間の背部採毛による，下毛＋分岐下毛の毛柄幅の統計量成績（背毛）

| 体重による区分 Classification by the body weight kg | 動物記号 Animal number | 柄幅の相加平均 ($\bar{x}$) Arithmetic mean of hair stalk $\mu$ | 標準偏差 (S) Standard deviation $\mu$ | 変異係数 $\frac{S}{\bar{x}}\times 100$ Coefficient of variation % |
|---|---|---|---|---|
| 7.5 | N—2.65K ♂ | 16.03 | 2.40 | 14.97 |
| | N—2.50K ♂ | 15.71 | 2.40 | 15.28 |
| | N—2.00K ♂ | 12.57 | 1.70 | 13.52 |
| | N—1.91K ♂ | 15.50 | 2.62 | 16.90 |
| | N—1.91K• ♂ | 12.30 | 1.64 | 13.33 |
| | N—2.36K ♀ | 13.59 | 2.24 | 16.48 |
| | N—2.08K ♀ | 14.72 | 2.61 | 17.73 |
| | N—2.03K ♀ | 12.70 | 1.48 | 11.65 |
| | N—2.00K ♀ | 14.62 | 2.47 | 16.89 |
| | N—1.84K ♀ | 12.33 | 1.84 | 14.92 |
| 5.5 | N—1.47K ♂ | 13.71 | 2.58 | 18.82 |
| | N—1.45K ♂ | 13.88 | 2.07 | 14.91 |
| | N—1.45K• ♂ | 12.63 | 1.46 | 11.56 |
| | N—1.45K•• ♂ | 14.93 | 2.19 | 14.69 |
| | N—1.44K ♂ | 14.02 | 2.03 | 14.48 |
| | N—1.46K ♀ | 14.82 | 1.96 | 13.23 |
| | N—1.45K ♀ | 12.29 | 2.25 | 18.31 |
| | N—1.42K ♀ | 14.45 | 1.94 | 13.43 |
| | N—1.41K ♀ | 13.67 | 2.21 | 16.17 |
| | N—1.40K ♀ | 13.51 | 1.83 | 13.55 |
| 3.5 | N—0.98K ♂ | 13.10 | 1.91 | 14.58 |
| | N—0.93K ♂ | 12.85 | 1.78 | 13.85 |
| | N—0.89K ♂ | 11.53 | 1.33 | 11.54 |
| | N—0.86K ♂ | 13.20 | 2.15 | 16.29 |
| | N—0.85K ♂ | 13.94 | 2.60 | 18.65 |
| | N—0.91K ♀ | 13.75 | 2.23 | 16.22 |
| | N—0.90K ♀ | 11.62 | 1.45 | 12.48 |
| | N—0.89K ♀ | 13.20 | 1.90 | 14.39 |
| | N—0.88K ♀ | 14.22 | 1.91 | 13.43 |
| | N—0.96K ♀ | 14.18 | 1.99 | 14.03 |

最初に牝牡別に各々体重区間について，次に牝牡性別の区間について検討した．

1. 体重別の差異（年齢別の差異）

(1) 牝獣の体重区間における柄幅の均等性は次のようである．

第 76 表

| | 体重区間 | | | 計及び平均 |
|---|---|---|---|---|
| | 3.5 kg | 5.5 kg | 7.5 kg | |
| | 11.62 μ | 14.82 μ | 12.33 μ | |
| | 13.20 | 14.45 | 14.72 | |
| | 14.18 | 13.67 | 13.59 | |
| | 14.22 | 13.51 | 12.70 | |
| | 13.75 | 12.29 | 14.62 | |
| 計 μ | 66.97 | 68.74 | 67.96 | 203.67 μ |
| 平均 μ | 13.37 | 13.74 | 13.59 | 13.57 μ |

$\Sigma x_i^2 = 2778.9055$

$N\bar{x}^2 = (13.57)^2 \times 15 = 2762.1735$

総平方和 $= \Sigma x_i^2 - N\bar{x}^2 = 16.732$

級内変動 $= 2778.9055 - \{(13.39)^2 + (13.74)^2 + (13.59)^2\} \times 5$

$= 2778.9055 - 2763.8390$

$(Sw) = 15.0665$

級間変動 $= 2763.8390 - 2762.1735$

$(Sb) = 1.6655$

第 77 表

| 要因 | 平方和 | 自由度 | 分散 |
|---|---|---|---|
| 級間変動 Sb | 1.6655 | 2 | 0.8325 |
| 級内変動 Sw | 15.0665 | 12 | 1.2555 |
| 総変動 | 16.732 | 14 | |

分散比 $= F_2^{12} = \dfrac{1.2555}{0.8325} = 1.5081$

解 イ. 仮説，体重別の区間の柄幅は均等であるとする.

ロ. 有意水準を 5 %とする.

ハ. 表により分散比 $F_{n2}^{n1} = F_2^{12}$ の棄却域は19.4

ニ. 計算による統計量は $F_2^{12} = 1.5081$ で棄却域にないから仮説は棄却出来ない故に有意な差があるといえない.

結果として体重差によつて下毛＋分岐下毛の柄幅に差異は認められないことになる. 牝の場合は観察値を通じて最小値は 3.5 kg 区間にあるが，最大値は 5.5 kg 区間にあつて常識的にも各区間に差異は認め難い.

(2) 牡獣の体重区間における柄幅の均等性は次のようである.

第　78　表

| | 体重区間 3.5 kg | 5.5 kg | 7.5 kg | 計及び平均 μ |
|---|---|---|---|---|
| | 12.85 μ | 14.02 μ | 12.57 μ | |
| | 13.10 | 13.71 | 15.71 | |
| | 13.20 | 13.88 | 15.50 | |
| | 13.94 | 12.63 | 12.30 | |
| | 11.53 | 14.93 | 16.03 | |
| 計　μ | 64.62 | 69.17 | 72.11 | 205.90 |
| 平均 μ | 12.92 | 13.83 | 14.42 | 13.73 |

$\Sigma x_i^2 = 2851.1476$

$N\bar{x}^2 = 188.2384 \times 15 = 2823.5760$

総平方和 $= \Sigma x_i^2 - N\bar{x}^2 = 2851.1476 - 2823.5760$

$= 27.5716$

級内変動 (within class variation)

$= 2851.1476 - \{(12.92)^2 + (13.83)^2 + (14.42)^2\} \times 5$

$= 2851.1476 - 2830.6585$

$= 20.4891$

級間変動 (between elass variation)

$= 2830.6585 - 2823.5750$

$= 7.0825$

第　79　表

| 要因 | 平方和 | 自由度 | 分散 |
|---|---|---|---|
| 級間変動 Sb | 7.0825 | 2 | 3.5412 |
| 級内変動 Sw | 20.4891 | 12 | 2.2976 |
| 総変動 | 27.5716 | 14 | |

分散比 $= F_{12}^2 = \dfrac{3.5412}{2.2976} = 1.5412$

解　イ．仮説，体重別の区間の柄幅は均等であるとする．

ロ．有意水準を 5 %とする．

ハ．表により分散比 $F_{n2}^{n1} = F_{12}^2$ の棄却域は 3.9

ニ．計算による統計量は $F_{12}^2 = 1.5412$ で棄却域にないから仮説は棄却出来ない故に有意な差があると言えない．

結果として体重差によつて下毛＋分岐下毛の柄幅には差異は認められないことになるが，観測値を通じて柄幅の最大値は 7.5 瓩区間にあり最小値は 3.5 瓩区間にあつて常識的には老大動物の被毛が比較的粗大に見える．

2.　性別の差異

さきに牝牡共にその統計量は体重区間には差異が認められないことになつたので，次に牝牡間の均等性を調査するためには，体重別には差異はないとして，これらを牝牡 2 区に分つて比較検討した．

第 80 表

| 牝 (♀) | | 牡 (♂) | |
|---|---|---|---|
| $x_i$ | $x_i^2$ | $x_i$ | $x_i^2$ |
| 13.59 | 184.6881 | 16.03 | 256.9609 |
| 14.72 | 216.6784 | 15.71 | 246.8041 |
| 12.70 | 161.2900 | 12.57 | 158.0049 |
| 14.62 | 213.7444 | 15.50 | 240.2500 |
| 12.33 | 152.0289 | 12.30 | 151.2900 |
| 14.82 | 219.6324 | 13.71 | 187.9641 |
| 12.29 | 151.0441 | 13.88 | 192.6544 |
| 14.45 | 208.8025 | 12.63 | 159.5169 |
| 13.67 | 186.8689 | 14.93 | 222.9049 |
| 13.51 | 182.5201 | 14.02 | 196.5604 |
| 13.75 | 189.0625 | 13.10 | 171.6100 |
| 11.62 | 135.0244 | 12.85 | 165.1225 |
| 13.20 | 174.2400 | 11.53 | 132.9409 |
| 14.22 | 202.2084 | 13.20 | 174.2400 |
| 14.18 | 201.0724 | 13.94 | 194.3236 |
| 平均 13.57 | $\Sigma x_i^2=2778.9055$ | 平均 13.72 | $\Sigma x_i^2=2851.1476$ |

$N\bar{x}^2=(13.57)^2\times 15$
$=2762.1735$
$\Sigma x_i^2-N\bar{x}^2=16.7320$

$N\bar{x}^2=(13.72)^2\times 15$
$=2823.576$
$\Sigma x_i^2-N\bar{x}^2=27.5716$

解 イ. 牝牡区間の柄幅は均等であるとする ($\sigma_1^2=\sigma_2^2=SP^2$ として $M_1=M_2$)

ロ. 有意水準を 5 %とする.

ハ. 統計量 $t=\dfrac{\bar{X}_1-\bar{X}_2}{SP\sqrt{1/N_1+1/N_2}}$ は自由度 $N_1+N_2-2=28$ の t 分布と仮定する.

ニ. 棄却域は表により $t>2.05$

ホ. 牡(♂)では $\bar{X}_1=13.72$, $\Sigma X_{1i}^2-N\bar{X}_1^2=27.5716$

牝(♀)では $\bar{X}_2=13.57$, $\Sigma X_{2i}^2-N\bar{X}_2^2=16.7320$

$$SP^2=\frac{27.5716+16.7320}{28}=1.5822$$

$$SP=1.2578$$

$$t=\frac{13.72-13.57}{1.2578\sqrt{\dfrac{2}{15}}}=\frac{0.15\sqrt{7.5}}{1.2578}=0.3265$$

これは棄却域にないから, 仮説は棄却出来ない. 故に有意な差があるといえない.

従つて背毛の下毛+分岐毛には牝牡別の差異は認められないことになる.

以上のように推計学的な処理では牝牡別及び体重別 (年齢別) で被毛の差異はないことになる.

## Ⅷ. 成獣被毛の季節的変化

齧歯類 (rodents) のもつとも定形的な換毛はカイウサギのように春秋2季に換毛して被

毛の性状が異り夏毛，冬毛と外観から確然と区別が出来る場合で，これに類するものはノウサギ，エチゴウサギ，北米の原野に棲む prairiedog (genus cynomys) 等である．

mouse のように年間通じて間断なく換毛しているがとくに秋季において（9月，10月）多量に換毛することを以つて秋季換毛 (autumnal molt) とする場合，又春秋2季（春季換毛 vernal molt）に同様な換毛が起る場合の記載もある。この際季節的に被毛の差異についての記述はない (COLLINS[9] 1923)．

1. 成獣毛量の年間変遷

筆者は Nutria で，成獣と認められる12ケ月以上の動物を月別に屠殺処分し，その生皮の㊢切片資料（8，9月の2ケ月採取不能）とくに皮脂腺位の切片を選んで5毛群を検鏡してえた成績（第91表）は，5毛群の全副側毛を単位として算出した結果である．この毛群の$\frac{1}{5}$に3原毛を加えたものがこれら毛皮の背毛の1毛群の代表数値とは言えない．皮脂腺位で最も完全に調整された調査の容易な毛群を選んだので生態変化の標準には差支ないが，毛群の大小，即ち毛量を測定するには適しない．なお福山地方の Nutria の飼育プールは屋外に煉瓦を積み上げて，ほとんど全部が流水の導入でなく，汲み込み式の水槽付で時には数日間換水を怠り気温の影響を受けるがままに放置してあるので，凡そ条件は一致しているといえよう．

第81表 (A)　背部毛群内の毛包の検鏡結果（5毛群）

動物記号 N—1

| 毛群番号 | (1) 中央毛包 先代毛 毛幹幅 a | b | c | 色調 その他 | 発育位置 | 発育記号 | 後継毛 毛幹幅 a | b | c | 色調 その他 | 発育位置 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | μ | μ | μ | | | | μ | μ | μ | | |
| 1 | 98.1 | 56.7 | 62.1 | 薄黒色，褐灰色，色素顆粒が皮質，髄質全面に散在する | 毛柄 hair stalk | ←S. Y. 単生青毛 | | | | | |
| 2 | 35.1 | 18.9 | 4.5 | 中心に髄質なく，黒色の皮質を乳濁色の毛小皮で楕円形に取巻く | 毛芒 hair awn | ←S. C. 単生幼毛 | | | | | |
| 3 | 40.5 | 26.1 | 髄質痕跡 | 基調色は乳濁色で，中央部に褐灰色の色素顆粒散在する | 毛根 hair root | ←S. O. 単生成毛 | | | | | |
| 4 | 4.5 | 4.5 | | 乳濁色で中心に褐灰色素顆粒点在す，円形 | 毛芒 hair awn | ←S. C. 単生幼毛 | | | | | |
| 5 | 31.5 | 29.7 | | 基調色は乳濁色で中央に褐灰色顆粒が点在する | 毛根 hair root | ←S. O. 単生成毛 | | | | | |
| | (2) 側毛包 | | | | | | | | | | |
| 1 | 26.1 | 26.1 | 8.1 | 薄黒色，皮質，髄質の全面に褐灰色顆粒散在す | 毛柄 hair stalk | ←S. Y. 単生青毛 | 4.5 | 4.5 | | 黒色，外輪の毛小皮乳濁色，円形 | 毛芒 hair awn |
| | 13.5 | 13.5 | | 乳濁色，円形 | 毛根 hair root | ←P.O.C.→ 成幼対生 | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 22.5 | 22.5 | 13.5 | 薄墨色 | 毛 柄 | ←S. Y. | | | | | | |
| | 1.89 | 18.9 | 11.7 | 薄墨色 | 毛 柄 | ←S. Y. | | | | | | |
| 3 | 17.1 | 17.1 | 4.5 | 薄墨色 | 毛 柄 | ←S. Y. | | | | | | |
| | 15.3 | 15.3 | | 乳濁色，円形 | 毛 根 | ←S. O. | | | | | | |
| 4 | 17.1 | 17.1 | | 乳濁色，円形 | 毛 根 | ←P.O.Y.→ | 31.5 | 29.7 | 8.1 | 薄墨色 | 毛 柄 | |
| | 26.1 | 24.3 | 11.7 | 薄墨色 | 毛 柄 | ←S. Y. | | | | | | |
| 5 | 13.5 | 13.5 | | 乳濁色，円形 | 毛 根 | ←P.O.Y.→ | 18.9 | 18.9 | 6.3 | 薄墨色 | 毛 柄 | |
| | 13.5 | 13.5 | | 乳濁色，円形 | 毛 根 | ←S. O. | | | | | | |

第81表 (B) (3) 副 側 毛

| 毛幹幅級間1.8μ代表値(μ) | 梶状毛(成毛) | | 発育毛 | | 成毛 | 青毛＋幼毛 | 総毛 |
|---|---|---|---|---|---|---|---|
| | 無色毛根 | 有色毛根 | 毛柄 | 毛芒, 毛葉, 毛頸 | O | Y＋C | O＋Y＋C |
| 2.7 | | | | 2 | | 2 | 2 |
| 4.5 | | | | 3 | | 3 | 3 |
| 6.3 | | | | 2 | | 2 | 2 |
| 8.1 | 1 | | | 4 | 1 | 4 | 5 |
| 9.9 | 145 | 2 | | 11 | 147 | 11 | 158 |
| 11.7 | 45 | | 7 | 6 | 45 | 13 | 58 |
| 13.5 | 2 | | 17 | 8 | 2 | 25 | 27 |
| 15.3 | 1 | | 6 | 2 | 1 | 8 | 9 |
| 17.1 | | | 2 | 4 | | 6 | 6 |
| 18.9 | | | 1 | 1 | | 2 | 2 |
| 20.7 | | | | 1 | | 1 | 1 |
| 22.5 | | | 1 | | | 1 | 1 |
| | 194 | 2 | 34 | 44 | 196 | 78 | 274 |

副側毛總数 C 44, Y 34 → 78; O 196; 78＋196 = 274

副側毛包總数 = 147 =
- 単生成毛 S. O. = Total O − {P. O. O. ＋T. O. O. (Y＋C)＋P. O. (Y＋C)}
  = 196 − {76＋24＋(77−12)}
  = 31
- 単生発育毛 S. (Y＋C) = S. Y. − S. C.
  = 78 − 77
  S. Y. = 1
- 対生毛束 P. O. (O＋Y＋C) = P. O. O ＋P. O. Y＋P. O. C
  = 65 ＋38 = 103
- 3連生毛束 T. O. O. (Y＋C) = T. O. O. Y＋T. O. O. C.
  = 12

第81表 (C)　(4)　副　側　毛　包

同一毛包に発生せる対生毛束及び連生毛束内の先代毛と後継毛との関係

| Oの根幅代表値(μ) / Y.Cの区別 / Y.C.毛幹幅の代表値(μ) | 8.1 | 9.9 | | 11.7 | | 13.5 | | 15.3 | | | | 計 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Y.C | Y. | C | Y. | C | Y. | C | Y. | C | Y | C | Y+C |
| 2.7 | | | 2 | | | | | | | | 2 | 2 |
| 4.5 | | | 2 | | 1 | | | | | | 3 | 3 |
| 6.3 | | | 1 | | 1 | | | | | | 2 | 2 |
| 8.1 | | | 4 | | | | | | | | 4 | 4 |
| 9.9 | | | 7 | | 4 | | | | | | 11 | 11 |
| 11.7 | 1 | 7 | 3 | | 2 | | | | | 7 | 6 | 13 |
| 13.5 | | 15 | 8 | 1 | | | | | | 16 | 8 | 24 |
| 15.3 | | 1 | 2 | 5 | | | | | | 6 | 2 | 8 |
| 17.1 | | 1 | 2 | | 2 | 1 | | | | 2 | 4 | 6 |
| 18.9 | | | 1 | | | | | 1 | | 1 | 1 | 2 |
| 20.7 | | | | | 1 | 1 | | | | | 1 | 1 |
| 22.5 | | | | | | | | | | 1 | | 1 |
| 計 | 1 | 24 | 32 | 6 | 11 | 2 | | 1 | | 33 | 44 | 77 |

P.O.C.+T.O.O.C.=44
P.O.Y+T.O.O.Y=33
P.O.O.=38
T.O.O.C.=, T.O.O.Y= } 12

第81表 (D)　成獣の背毛群毛包の分類（5毛群）

動物記号 N—1 背毛

| 毛の発育記号 | 3 原毛包 中央毛包量 No. | 側毛包量 No. | 毛包量合計 No. | % | 毛量合計 No. | 副側毛包 毛包量 No. | % | 毛量 No. | % | 総毛包量 No. | % | 総毛量 No. | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC. | 2 | | 2 | | C. 3 | | | C. 34 | 12.4 | 2 | | C. 37 | 12.7 |
| SY. | 1 | 5 | 6 | | Y. 8 | 1 | 0.7 | Y. 44 | 16.1 | 7 | | Y. 52 | 17.8 |
| SO. | 2 | 2 | 4 | | O. 7 | 31 | 21.1 | O. 196 | 71.5 | 35 | | O. 203 | 69.5 |
| P.O.C. | | 1 | 1 | | | } 65 | 44.2 | | | } 68 | | | |
| P.O.Y. | | 2 | 2 | | | | | | | | | | |
| O.O.P. | | | | | | 38 | 25.9 | | | 38 | | | |
| T.O.O.C. | | | | | | } 12 | 8.2 | | | } 12 | | | |
| T.O.O.Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 18 | 147 | 100.1 | 274 | 100 | 162 | | 292 | 100 |

第81表 (E)　各態生育毛量の毛包量に対する比率

| 副側毛 | | |
|---|---|---|
| 毛包量 | 147 | 100 % |
| 幼毛量 | 44 | 29.9 |
| 青毛量 | 34 | 23.1 |
| 成毛量 | 196 | 133.3 |
| 毛量 | 274 | 186.4 |

第 82表 成獣の背毛群毛包の分類（5毛群）

動物記号 N—2 背毛

| 毛の発育記号 | 3 原毛包 中央毛包量 No. | 3 原毛包 側毛包量 No. | 3 原毛包 毛包量合計 No. | 3 原毛包 % | 3 原毛包 毛量合計 No. | 副側毛包 毛包量 No. | 副側毛包 毛包量 % | 副側毛包 毛量 No. | 副側毛包 毛量 % | 総毛包量 No. | 総毛包量 % | 総毛量 No. | 総毛量 % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC. | 2 | 1 | 3 | | C. 3 | | | C. 37 | 10.3 | 3 | | C. 40 | 10.8 |
| SY. | | 4 | 4 | | Y. 4 | 18 | 9.1 | Y. 84 | 23.6 | 22 | | Y. 88 | 23.7 |
| SO. | 3 | 5 | 8 | | O. 8 | 29 | 14.6 | O. 235 | 66.0 | 37 | | O. 243 | 65.5 |
| P. O. C. | | | | | | } 96 | 48.5 | | | } 96 | | | |
| P. O. Y. | | | | | | | | | | | | | |
| P. O. O. | | | | | | 48 | 24.2 | | | 48 | | | |
| T. O. O. C. | | | | | | } 7 | 35 | | | } 7 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 15 | 198 | 99.9 | 356 | 99.9 | 213 | | 371 | 100 |

第 82 表続 各態生育毛量の毛包量に対する比率

| 副側毛 | | |
|---|---|---|
| 毛包量 | 198 | 100 % |
| 幼毛量 | 37 | 18.6 |
| 青毛量 | 84 | 42.4 |
| 成毛量 | 235 | 118.7 |
| 毛量 | 356 | 179.8 |

第 83表 成獣の背毛群毛包の分類（5毛群）

動物記号 N—3 背毛

| 毛の発育記号 | 3 原毛包 中央毛包量 No. | 3 原毛包 側毛包量 No. | 3 原毛包 毛包量合計 No. | 3 原毛包 % | 3 原毛包 毛量合計 No. | 副側毛包 毛包量 No. | 副側毛包 毛包量 % | 副側毛包 毛量 No. | 副側毛包 毛量 % | 総毛包量 No. | 総毛包量 % | 総毛量 No. | 総毛量 % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC. | | 1 | 1 | | C. 8 | | | C. 74 | 17.3 | 1 | | C. 82 | 18.2 |
| SY. | | 1 | 1 | | Y. 3 | | | Y. 45 | 10.5 | 1 | | Y. 48 | 10.6 |
| SO. | 2 | 2 | 4 | | O. 13 | 21 | 9.9 | O. 308 | 72.1 | 25 | | O. 321 | 71.2 |
| P. O. C. | 3 | 4 | 7 | | | } 95 | 44.8 | | | } 104 | | | |
| P. O. Y. | | 2 | 2 | | | | | | | | | | |
| P. O. O. | | | | | | 72 | 34.0 | | | 72 | | | |
| T. O. O. C. | | | | | | } 24 | 11.3 | | | } 24 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 24 | 212 | 100 | 427 | 99.9 | 227 | | 451 | 100 |

第 83 表続 各態生育毛量の毛包量に対する比率

| 副側毛 | | |
|---|---|---|
| 毛包量 | 212 | 100 % |
| 幼毛量 | 74 | 34.9 |
| 青毛量 | 45 | 21.2 |
| 成毛量 | 308 | 145.3 |
| 毛量 | 427 | 201.4 |

第84表　成獣の背毛群毛包の分類（5毛群）

動物記号 N—4 背毛

| 毛の発育記号 | 3原毛包 中央毛包量 No. | 3原毛包 側毛包量 No. | 3原毛包 毛包量合計 No. | 3原毛包 % | 3原毛包 毛量合計 No. | 副側毛包 毛包量 No. | 副側毛包 毛包量 % | 副側毛包 毛量 No. | 副側毛包 毛量 % | 総毛包量 No. | 総毛包量 % | 総毛量 No. | 総毛量 % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC. | 4 | 6 | 10 | | C. 10 | 8 | 5.9 | C. 45 | | 19 | | C. 55 | 21.0 |
| SY. | | 1 | 1 | | Y. 1 | | | Y. 48 | | | | Y. 49 | 18.7 |
| SO. | 1 | 1 | 2 | | O. 2 | 20 | 14.7 | O. 156 | | 22 | | O. 158 | 60.3 |
| P. O. C. | | | | | | 80 | 58.8 | | | 80 | | | |
| P. O. Y. | | | | | | | | | | | | | |
| P. O. O. | | | | | | 23 | 16.9 | | | 23 | | | |
| T. O. O. C. | | | | | | 5 | 3.7 | | | 5 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 8 | 13 | | 13 | 136 | 100 | 249 | | 149 | | 262 | 100 |

第84表続　各態生育毛量の毛包量に対する比率

| 副側毛 | | | | |
|---|---|---|---|---|
| 毛包量 | | | 136 | 100 % |
| 幼毛量 | | | 45 | 33.1 |
| 青毛量 | | | 48 | 35.3 |
| 成毛量 | | | 156 | 114.7 |
| 毛量 | | | 249 | 183.1 |

第85表　成獣の背毛群毛包の分類（5毛群）

動物記号 N—5 背毛

| 毛の発育記号 | 3原毛包 中央毛包量 No. | 3原毛包 側毛包量 No. | 3原毛包 毛包量合計 No. | 3原毛包 % | 3原毛包 毛量合計 No. | 副側毛包 毛包量 No. | 副側毛包 毛包量 % | 副側毛包 毛量 No. | 副側毛包 毛量 % | 総毛包量 No. | 総毛包量 % | 総毛量 No. | 総毛量 % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. C. | | | | | C. 3 | 3 | 1.3 | C. 66 | 15.7 | 7 | | C. 69 | 15.6 |
| S. Y. | 4 | | 4 | | Y. 7 | | | Y. 52 | 12.4 | | | Y. 59 | 13.4 |
| S. O. | 1 | 4 | 5 | | O. 11 | 52 | 22.3 | O. 302 | 71.9 | 57 | | O. 313 | 71.0 |
| P. O. C. | | 3 | 3 | | | 106 | 45.5 | | | 112 | | | |
| P. O. Y. | | 3 | 3 | | | | | | | | | | |
| P. O. O. | | | | | | 63 | 27.0 | | | 63 | | | |
| T. O. O. C. | | | | | | 9 | 3.9 | | | 9 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 21 | 233 | 100 | 420 | 100 | 248 | | 441 | 100 |

第85表続　各態生育毛量の毛包量に対する比率

| 副側毛 | | | | |
|---|---|---|---|---|
| 毛包量 | | | 233 | 100 % |
| 幼毛量 | | | 66 | 28.3 |
| 青毛量 | | | 52 | 22.3 |
| 成毛量 | | | 302 | 129.6 |
| 毛量 | | | 420 | 180.3 |

第86表 成獣の背毛群毛包の分類（5毛群）

動物記号 N—6 背毛

| 毛の発育記号 | 3 原毛包 中央毛包量 No. | 側毛包量 No. | 毛包量合計 No. | % | 毛量合計 No. | 副側毛包 毛包量 No. | % | 毛量 No. | % | 総毛包量 No. | % | 総毛量 No. | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. C. | | 1 | 1 | | C. 2 | | | C. 52 | 19.6 | 1 | | C. 54 | 19.2 |
| S. Y. | 3 | 2 | 5 | | Y. 5 | 61 | 30.0 | Y. 67 | 25.3 | 66 | | Y. 72 | 25.6 |
| S. O. | 2 | 6 | 8 | | O. 9 | 82 | 40.4 | O. 146 | 55.1 | 90 | | O. 155 | 55.2 |
| P. O. C. | | 1 | 1 | | | 50 | 24.6 | | | 51 | | | |
| P. O. Y. | | | | | | 6 | 3.0 | | | 6 | | | |
| P. O. O. | | | | | | 2 | 1.0 | | | 2 | | | |
| T. O. O. C. | | | | | | 2 | 1.0 | | | 2 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 16 | 203 | 100 | 265 | 100 | 218 | | 281 | 100 |

第86表続 各態生育毛量の毛包量に対する比率

| 副側毛 | | |
|---|---|---|
| 毛包量 | 203 | 100 % |
| 幼毛量 | 52 | 25.6 |
| 青毛量 | 67 | 33.0 |
| 成毛量 | 146 | 71.9 |
| 毛量 | 265 | 130.5 |

第87表 (A) 背部毛群内の毛包の検鏡結果（5毛群）

動物記号 N—7

(1) 中央毛包

| 毛群番号 | 先代毛 毛幹幅 a (μ) | b (μ) | c (μ) | 色調その他 | 発育位置 | 発育記号 | 後継毛 毛幹幅 a (μ) | b (μ) | c (μ) | 色調その他 | 発育位置 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 31.5 | 29.7 | | 基調色は乳濁色で中央に褐灰色顆粒点在す | 毛根 | ←P. O. C→ | 71.1 | 35.1 | 44.1 | 基調色は赤黄色で黄色素顆粒が皮質髄質に散在す | 毛葉葉明帯 |
| 2 | 152.1 | 62.1 | 89.1 | 濃褐灰色，皮質髄質に褐灰色顆粒散在す | 暗毛葉下位 | ←S. C. | | | | | |
| 3 | 63.9 | 56.7 | | 基調色は乳濁色で中央に褐灰色顆粒点在す | 毛根 | ←S. O. | | | | | |
| 4 | 215.1 | 89.1 | 143.1 | 濃褐灰色，褐灰色顆粒が皮質，髄質に充満す | 暗毛葉 | ←S. C. | | | | | |
| 5 | 173.7 | 71.1 | 117.9 | 基調色は瀰漫性の黄色で黄色素顆粒が皮質，髄質に散在す | 毛葉葉明帯 | ←S. C. | | | | | |

（2）　側　毛　包

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36.9 | 29.7 | | 基調色は乳濁色で中央に褐灰色顆粒が点在す | 毛　根 | ←S. O. | | | | | | |
| | 58.5 | 26.1 | 31.5 | 濃褐灰色，皮質，髄質に色素顆粒充満す | 毛　頸 | ←S. C. | | | | | | |
| 2 | 27.9 | 27.9 | | 基調色乳濁色中央に褐灰色顆粒点在す | 毛　根 | ←S. O. | | | | | | |
| | 29.7 | 26.1 | | 同　　上 | 毛　根 | ←S. O. | | | | | | |
| 3 | 17.1 | 17.1 | | 基調色乳濁色色素顆粒なし | 毛　根 | ←S. O. | | | | | | |
| | 22.5 | 17.1 | | 同　　上 | 毛　根 | ←S. O. | | | | | | |
| 4 | 80.1 | 42.3 | 44.1 | 濃褐灰色，皮質，髄質に褐灰色顆粒充満す | 毛　頸 | ←S. C. | | | | | | |
| | 125.1 | 53.1 | 83.7 | 薄墨色，皮質，髄質に褐灰色素顆粒散在す | 毛　柄 | ←S. Y. | | | | | | |
| 5 | 26.1 | 26.1 | | 乳濁色， | 毛　根 | ←P.O.Y.→ | 22.5 | 17.1 | | 黒色の皮質を乳濁色の毛小皮で取巻く | 毛　芒 | |
| | 35.1 | 20.7 | | 黒色皮質を乳濁色の毛小皮で取巻く | 毛　芒 | ←S. C. | | | | | | |

第87表（B）　（3）　副　側　毛

| 毛幹幅級間 1.8μ 代表値（μ） | 棍状毛（成毛） | | 発育毛 | | 成毛 O | 青毛＋幼毛 Y＋C | 総毛 O＋Y＋C |
|---|---|---|---|---|---|---|---|
| | 無色毛根 | 有色毛根 | 毛柄 | 毛芒，毛葉，毛頸 | | | |
| 4.5 | | | | 2 | | 2 | 2 |
| 6.3 | | | | 4 | | 4 | 4 |
| 8.1 | 1 | | | 9 | 1 | 9 | 10 |
| 9.9 | 52 | 5 | | 9 | 57 | 9 | 66 |
| 11.7 | 94 | 16 | 8 | 11 | 110 | 19 | 129 |
| 13.5 | 14 | 6 | 19 | 11 | 20 | 30 | 50 |
| 15.3 | | 3 | 25 | 7 | 3 | 32 | 35 |
| 17.1 | | | 11 | 4 | | 15 | 15 |
| 18.9 | | | 3 | 4 | | 7 | 7 |
| 20.7 | 1 | 1 | 3 | 2 | 2 | 5 | 7 |
| 22.5 | 1 | | | 1 | 1 | 1 | 2 |
| 24.3 | | | | | | | |
| 26.1 | | 1 | 1 | | 1 | 1 | 2 |
| 27.9 | | | | 1 | | 1 | 1 |
| 29.7 | | | | | | | |
| 31.5 | | | | | | | |
| 33.3 | | | 1 | | | 1 | 1 |
| 計 | 163 | 32 | 71 | 65 | 195 | 136 | 331 |

副側毛総数 C. 65, Y. 71 → 136; O. 195; 136 + 195 = 331

副側毛包総数 211 =
- 単生成毛　S. O. = Total O − PO(Y+C) = 195 − 120 = 75
- 単生発育毛　S(Y+C) = S. Y + S. C. = 136 − 120 = 16
- 対生毛束 PO(O+Y+C) = PO. O + P. O. Y. + P. O. C. = 120
  （P. O. Y. + P. O. C. = 120）

第87表 (C)　(4)　副側毛包

同一毛包に発生せる対生毛束及び連生毛束内の先代毛と後継毛との関係

| O の根幅代表値 (μ) | 9.9 | | 11.7 | | 13.5 | | | 26.1 | | | | 計 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y. C の区別 / Y. C. 毛幹幅, 代表値 (μ) | Y | C | Y | C | Y | C | | Y | C | Y | C | Y+C |
| 4.5 | | 1 | | 1 | | | | | | | 2 | 2 |
| 6.3 | | 1 | | 2 | | 2 | | | | | 5 | 5 |
| 8.1 | | 2 | | 6 | | | | | | | 8 | 8 |
| 9.9 | | 3 | | 6 | | 1 | | | | | 10 | 10 |
| 11.7 | 2 | 5 | 4 | 6 | | | | | | 6 | 11 | 17 |
| 13.5 | 6 | 4 | 10 | 7 | | | | | | 16 | 11 | 27 |
| 15.3 | 7 | 1 | 15 | 6 | | | | | | 22 | 7 | 29 |
| 17.1 | | 1 | 7 | 3 | 1 | | | | | 8 | 4 | 12 |
| 18.9 | 1 | | | 2 | 1 | 2 | | | | 2 | 4 | 6 |
| 20.7 | | | | 1 | 1 | | | a. 20.7 / b. 11.7 | 1 | 1 | 2 | 3 |
| 22.5 | | | | 1 | | | | | | | 1 | 1 |
| | 16 | 18 | 36 | 41 | 3 | 5 | | | 1 | 55 | 65 | 120 |

P. O. O. = O　　P. O. C. = 65　　P. O. Y. = 55

第87表　(D) 成獣の背毛群毛包の分類（5毛群）

動物記号 N—7 背毛

| 毛の発育記号 | 3 原毛包 | | | | | 副側毛包 | | | | 総毛包量 | | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 No. | 側毛包量 No. | 毛包量合計 No. | % | 毛量合計 No. | 毛包量 No. | % | 毛量 No. | % | No. | % | No. | % |
| S. C. | 3 | 3 | 6 | | C. 8 | | | C. 65 | 19.6 | 6 | | C. 73 | 21.0 |
| S. Y. | | 1 | 1 | | Y. 1 | 16 | 7.6 | Y. 71 | 21.5 | 17 | | Y. 72 | 20.7 |
| S. O. | 1 | 5 | 6 | | O. 8 | 75 | 35.5 | O. 195 | 58.9 | 81 | | O. 203 | 58.3 |
| P. O. C. | 1 | 1 | 2 | | | 65 | 30.8 | | | 67 | | | |
| P. O. Y. | | | | | | 55 | 26.1 | | | 55 | | | |
| P. O. O. | | | | | | | | | | | | | |
| T. O. O. C. | | | | | | | | | | | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 17 | 211 | 100 | 331 | 100 | 226 | | 348 | 100 |

第87表（E）　各態生育毛量の毛包量に対する比率

| 副側毛 | | | |
|---|---|---|---|
| 毛 | 包 | 量 | 211 | 100 % |
| 幼 | 毛 | 量 | 65 | 30.8 |
| 青 | 毛 | 量 | 71 | 33.6 |
| 成 | 毛 | 量 | 195 | 92.4 |
| 毛 | | 量 | 331 | 156.8 |

第88表　成獣の背毛群毛包の分類（5毛群）

動物記号 N—10 背毛

| 毛の発育記号 | 3 原毛包 | | | | | 副側毛包 | | | | 総毛包量 | | 総毛量 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 中央毛包量 No. | 側毛包量 No. | 毛包量合計 No. | % | 毛量合計 No. | 毛包量 No. | % | 毛量 No. | % | No. | % | No. | % |
| S. C. | 1 | | 1 | | C. 8 | 2 | 0.9 | C. 28 | 8.6 | 4 | | C. 36 | 10.3 |
| S. Y. | 1 | | 1 | | Y. 2 | | | Y. 46 | 14.1 | | | Y. 48 | 13.8 |
| S. O. | 2 | 3 | 5 | | O. 13 | 119 | 54.8 | O. 252 | 77.3 | 124 | | O. 265 | 75.9 |
| P. O. C. | 1 | 6 | 7 | | | 57 | 26.3 | | | 65 | | | |
| P. O. Y. | | 1 | 1 | | | | | | | | | | |
| P. O. O. | | | | | | 24 | 11.1 | | | 24 | | | |
| T. O. O. C. | | | | | | 15 | 6.9 | | | 15 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 23 | 217 | 100 | 326 | 100 | 232 | | 349 | 100 |

第88表続　各態生育毛量が毛包量に対する比率

| 副側毛 | | | |
|---|---|---|---|
| 毛 | 包 | 量 | 217 | 100 % |
| 幼 | 毛 | 量 | 28 | 12.9 |
| 青 | 毛 | 量 | 46 | 21.2 |
| 成 | 毛 | 量 | 252 | 116.1 |
| 毛 | | 量 | 326 | 150.2 |

第89表 成獣の背毛群毛包の分類（5毛群）

動物記号 N—11 背毛

| 毛の発育記号 | 3 原毛包 中央毛包量 No. | 側毛包量 No. | 毛包量合計 No. | % | 毛量合計 No. | 副側毛包 毛包量 No. | % | 毛量 No. | % | 総毛包量 No. | % | 総毛量 No. | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. C. | 4 | 2 | 6 | | C. 9 | | | C. 71 | | 6 | | C. 80 | |
| S. Y. | | 1 | 1 | | Y. 4 | 8 | 3.7 | Y. 74 | | 9 | | Y. 78 | |
| S. O. | 1 | 1 | 2 | | O. 8 | 36 | 16.8 | O. 247 | | 38 | | O. 255 | |
| P. O. C. | | 3 | 3 | | | } 129 | 60.3 | | | } 135 | | | |
| P. O. Y. | | 3 | 3 | | | | | | | | | | |
| P. O. O. | | | | | | 33 | 15.4 | | | 33 | | | |
| T. O. O. C. | | | | | | } 8 | 3.7 | | | } 8 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 10 | 15 | | 21 | 214 | 99.9 | 392 | | 229 | | 413 | |

第89表続 各態生育毛量が毛包量に対する比率

| 副側毛 | | |
|---|---|---|
| 毛包量 | 214 | 100 % |
| 幼毛量 | 71 | 33.2 |
| 青毛量 | 74 | 34.6 |
| 成毛量 | 247 | 115.6 |
| 毛量 | 392 | 183.2 |

第90表 成獣の背毛群毛包の分類（5毛群）

動物記号 N—12 背毛

| 毛の発育記号 | 3 原毛包 中央毛包量 No. | 側毛包量 No. | 毛包量合計 No. | % | 毛量合計 No. | 副側毛包 毛包量 No. | % | 毛量 No. | % | 総毛包量 No. | % | 総毛量 No. | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. C. | 2 | | 2 | | C. 7 | 1 | 0.3 | C. 50 | | 3 | | C. 57 | |
| S. Y. | 1 | | 1 | | Y. 1 | 39 | 12.4 | Y. 145 | | 40 | | Y. 146 | |
| S. O. | 1 | 5 | 6 | | O. 11 | 60 | 19.0 | O. 378 | | 66 | | O. 389 | |
| P. O. C. | 1 | 4 | 5 | | | } 112 | 35.6 | | | } 117 | | | |
| P. O. Y. | | | | | | | | | | | | | |
| P. O. O. | | | | | | 60. | 19.0 | | | 60 | | | |
| T. O. O. C. | | | | | | } 43 | 13.7 | | | } 43 | | | |
| T. O. O. Y. | | | | | | | | | | | | | |
| | 5 | 9 | 14 | | 19 | 315 | 100 | 573 | | 329 | | 592 | |

第90表続 各態生育毛量が毛包量に対する比率

| 副側毛 | | |
|---|---|---|
| 毛包量 | 315 | 100 % |
| 幼毛量 | 50 | 15.9 |
| 青毛量 | 145 | 46.0 |
| 成毛量 | 378 | 120.0 |
| 毛量 | 573 | 181.9 |

第91表　副側毛における毛包量と発生過程各相の毛量との比率の月別比較

（成獣の副側毛の場合）

| 動物記号 | 区別 / 月別 | 毛包（毛母基） | | 全副側毛 O+Y+C | | 成毛（棍状毛） O | | 青毛 Y | | 幼毛 C | | 全発育毛 Y+C | | 成，青毛 O+Y | | 成，成，発育毛連生毛包 T.O.O.Y. | | 成，成毛対生毛包 P.O.O. | | T.O.O.Y.+P.O.O. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| N—1 | 1 月 | 147 | 100 | 274 | 186.4 | 196 | 133.3 | 34 | 23.1 | 44 | 29.9 | 78 | 53.0 | 230 | 156.4 | 12 | 8.2 | 38 | 25.9 | 50 | 34.1 |
| N—2 | 2 月 | 198 | 100 | 356 | 179.8 | 235 | 118.7 | 84 | 42.4 | 37 | 18.6 | 121 | 61.1 | 319 | 161.1 | 7 | 3.5 | 48 | 24.2 | 55 | 27.8 |
| (N—B) N—3 | 3 月 | 212 | 100 | 427 | 201.4 | 308 | 145.3 | 45 | 21.2 | 74 | 34.9 | 119 | 56.1 | 353 | 166.5 | 24 | 11.3 | 72 | 34.0 | 96 | 45.3 |
| N—4 | 4 月 | 136 | 100 | 249 | 183.1 | 156 | 114.7 | 48 | 35.3 | 45 | 33.1 | 93 | 68.4 | 204 | 150.0 | 5 | 3.7 | 23 | 16.9 | 28 | 20.6 |
| N—5 | 5 月 | 233 | 100 | 420 | 180.3 | 302 | 129.6 | 52 | 22.3 | 66 | 28.3 | 118 | 50.6 | 354 | 151.9 | 9 | 3.9 | 63 | 27.0 | 72 | 30.9 |
| N—6 | 6 月 | 203 | 100 | 265 | 130.5 | 146 | 71.9 | 67 | 33.0 | 52 | 25.6 | 119 | 58.6 | 213 | 104.9 | 2 | 1.0 | 2 | 1.0 | 4 | 2.0 |
| N—7 | 7 月 | 211 | 100 | 331 | 156.8 | 195 | 92.4 | 71 | 33.6 | 65 | 30.8 | 136 | 64.5 | 266 | 126.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| N—10 | 10 月 | 217 | 100 | 326 | 150.2 | 252 | 116.1 | 46 | 21.2 | 28 | 12.9 | 74 | 34.1 | 298 | 137.3 | 15 | 6.9 | 24 | 11.1 | 39 | 18.0 |
| N—11 | 11 月 | 214 | 100 | 392 | 183.2 | 247 | 115.4 | 74 | 34.6 | 71 | 33.2 | 145 | 67.8 | 321 | 150.0 | 8 | 3.7 | 33 | 15.4 | 41 | 19.2 |
| N—12 | 12 月 | 315 | 100 | 573 | 181.9 | 378 | 120.0 | 145 | 46.0 | 50 | 15.9 | 195 | 61.9 | 523 | 166.0 | 43 | 13.7 | 60 | 19.0 | 103 | 32.7 |

年間の毛量変遷の曲線作図

年間を通じて月別と毛量との関係式を近似2次式（抛物線の方程式）$Y=a+bx+cx^2$ として，最小自乗法による次の規準方程式で解く．

$$\begin{cases}\Sigma(n)a+\Sigma(x)b+\Sigma(x^2)c=\Sigma(y)\\ \Sigma(x)a+\Sigma(x^2)b+\Sigma(x^3)c=\Sigma(xy)\\ \Sigma(x^2)a+\Sigma(x^3)b+\Sigma(x^4)c=\Sigma(x^2y)\end{cases}$$

又一部は近似一次式（直線の方程式）

$Y=a+bx$ として，最小自乗法による次の規準方程式で解く．

$$\begin{cases}\Sigma(n)a+\Sigma(x)b=\Sigma(y)\\ \Sigma(x)a+\Sigma(x^2)b=\Sigma(xy)\end{cases}$$

第 92 表

| x | a | bx | $cx^2$ | Y（期待値） | |
|---|---|---|---|---|---|
| | | | | | 4 捨 5 入 |
| 1 | 212.952 | − 14.645 | + 0.985 | 199.292 | 199.3 |
| 2 | 〃 | − 29.290 | + 3.940 | 187.602 | 187.6 |
| 3 | 〃 | − 43.935 | + 8.865 | 177.882 | 177.9 |
| 4 | 〃 | − 58.580 | + 15.760 | 170.132 | 170.1 |
| 5 | 〃 | − 73.225 | + 24.625 | 164.352 | 164.4 |
| 6 | 〃 | − 87.870 | + 35.460 | 160.542 | 160.5 |
| 7 | 〃 | −102.515 | + 48.265 | 158.702 | 158.7※ |
| 8 | 〃 | −117.160 | + 63.040 | 158.832 | 158.8 |
| 9 | 〃 | −131.805 | + 79.785 | 160.932 | 160.9 |
| 10 | 〃 | −146.450 | + 98.500 | 165.002 | 165.0 |
| 11 | 〃 | −161.095 | +119.185 | 171.042 | 171.0 |
| 12 | 〃 | −175.740 | +141.840 | 179.052 | 179.1 |

Y を微分して極小を求めれば

$$Y=212.952-14.645x+0.985x^2\cdots\cdots(1)$$

$$Y'=-14.645+(0.985\times2)x=0$$

$$-14.645+1.97x=0$$

$$x=7.434\cdots\cdots(2)$$

(1) に (2) を代入せば $Y=158.5164$ となり，

4 捨 5 入して

※ 極小は $\begin{cases}x=7.4\\ Y=158.5\end{cases}$

同様な方法により，(2) O 棍状毛（成毛）の場合，及び (3) O+Y 棍状毛と青毛混合の場合は次のようである．

(1) O+Y+C 総毛の場合

規準方程式の係数の計算を行なえば次のようである．

第　9 3　表

| x | n | x | $x^2$ | $x^3$ | $x^4$ | (観察値) y | xy | $x^2y$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1 | 186.4 | 186.4 | 186.4 |
| 2 | 1 | 2 | 4 | 8 | 16 | 179.8 | 359.6 | 719.2 |
| 3 | 1 | 3 | 9 | 27 | 81 | 201.4 | 604.2 | 1812.6 |
| 4 | 1 | 4 | 16 | 64 | 256 | 183.1 | 732.4 | 2929.6 |
| 5 | 1 | 5 | 25 | 125 | 625 | 180.3 | 901.5 | 4507.5 |
| 6 | 1 | 6 | 36 | 216 | 1296 | 130.5 | 783.0 | 4698.0 |
| 7 | 1 | 7 | 49 | 343 | 2401 | 156.8 | 1097.6 | 7683.2 |
| 10 | 1 | 10 | 100 | 1000 | 10000 | 150.2 | 1502.0 | 15020.0 |
| 11 | 1 | 11 | 121 | 1331 | 14641 | 183.2 | 2015.2 | 22167.2 |
| 12 | 1 | 12 | 144 | 1728 | 20736 | 181.9 | 2182.8 | 26193.6 |
|  | 10 | 61 | 505 | 4843 | 50053 | 1733.6 | 10364.7 | 85917.3 |

従つて規準方程式は次のようである.

$$\begin{cases} 10a+61b+505c=1733.6 & \cdots\cdots(1) \\ 61a+505b+4843c=10364.7 & \cdots\cdots(2) \\ 505a+4843b+50053c=85917.3 & \cdots\cdots(3) \end{cases}$$

これを解けば, $a=212.952$

$b=-14.645$

$c=0.985$

近似2次式は $Y=a+bx+cx^2$

よつて $Y=212.952-14.645x+0.985x^2$

第　9 4　表

| (2)　Oの場合 | | | (3)　O+Yの場合 | | |
|---|---|---|---|---|---|
| 月別 (x) | 観察値 (y) | 期待値 (Y) | 月 (x) | 観察値 (y) | 期待値 (Y) |
| 1 | 133.3 | 140.9 | 1 | 156.4 | 172.0 |
| 2 | 118.7 | 129.5 | 2 | 161.1 | 159.3 |
| 3 | 145.3 | 120.0 | 3 | 166.5 | 149.0 |
| 4 | 114.7 | 112.4 | 4 | 150.0 | 140.9 |
| 5 | 129.6 | 106.8 | 5 | 151.9 | 135.2 |
| 6 | 71.9 | 103.1 | 6 | 104.9 | 131.8 |
| 7 | 92.4 | 101.4 | 7 | 126.1 | 130.7 |
| 8 |  | 101.6※ | 8 |  | 132.0 |
| 9 |  | 103.7 | 9 |  | 135.5 |
| 10 | 116.1 | 107.8 | 10 | 137.3 | 141.4 |
| 11 | 115.4 | 113.8 | 11 | 150.0 | 149.6 |
| 12 | 120.0 | 121.7 | 12 | 166.0 | 160.1 |
| ※極小 $\begin{cases} x=7.4 \\ Y=101.2 \end{cases}$ | | | ※極小 $\begin{cases} x=7.0\ (6.966 \quad 4\text{捨}5\text{入}) \\ Y=130.7 \end{cases}$ | | |

(4) Y+C 青色と幼毛の場合

規準方程式の係数の計算を行なえば

第 95 表

| 月 別 (x) | n | x | $x^2$ | 観 察 値 (y) | xy |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 53.0 | 53.0 |
| 2 | 1 | 2 | 4 | 61.1 | 122.2 |
| 3 | 1 | 3 | 9 | 56.1 | 168.3 |
| 4 | 1 | 4 | 16 | 68.4 | 273.6 |
| 5 | 1 | 5 | 25 | 50.6 | 253.0 |
| 6 | 1 | 6 | 36 | 58.6 | 351.6 |
| 7 | 1 | 7 | 49 | 64.5 | 451.5 |
| 10 | 1 | 10 | 100 | 34.1 | 341.0 |
| 11 | 1 | 11 | 121 | 67.8 | 745.8 |
| 12 | 1 | 12 | 144 | 61.9 | 742.8 |
| | 10 | 61 | 505 | 576.1 | 3502.8 |

従つて規準方程式は

$$\begin{cases} 10\,a+61\,b=576.1 \\ 61\,a+505\,b=3502.8 \end{cases}$$

これを解けば

$$\begin{cases} a=58.1337 \\ b=-0.08585 \end{cases}$$

近似式は直線の方程式で

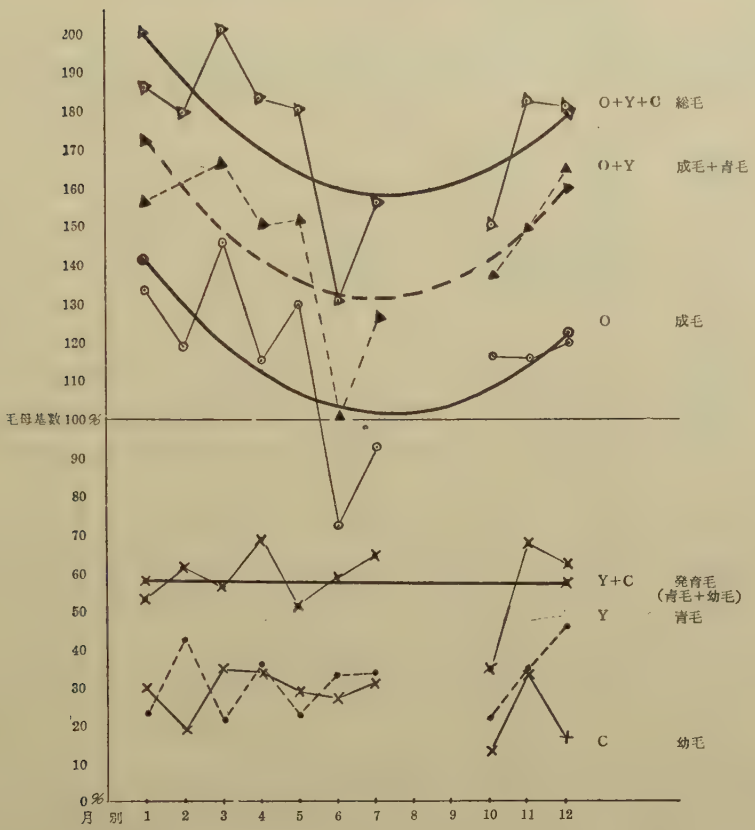
$$Y=a+bx$$

$$Y=58.1337-0.08585\,x$$

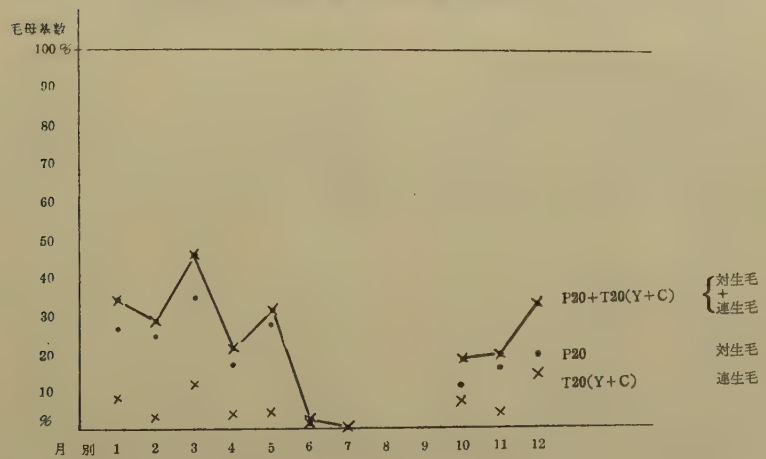
第 96 表

| x (月 別) | Y (期待値) |
|---|---|
| 1 | 58.0 |
| 2 | 57.96 |
| 3 | 57.88 |
| 4 | 57.79 |
| 5 | 57.70 |
| 6 | 57.62 |
| 7 | 57.53 |
| 8 | 57.45 |
| 9 | 57.36 |
| 10 | 57.28 |
| 11 | 57.19 |
| 12 | 57.10 |

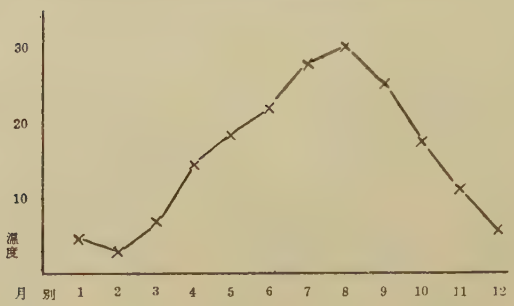
第 46 図　成獣毛量の年間変遷（副側毛）（A）




第 47 図　成獣毛量の月間変遷（副側毛）（B）



第 48 図　福山市の月別平均気温図（1947年）




考察，この調査成績によると年間通じて青毛，幼毛の発育毛の発生差はない（第91表）．温暖時，暑熱時は成毛の落毛が早く，下毛の場合は後継毛が青毛期の内にそれ等と対生している成毛の多くが落毛するので，成毛2個の対生することは稀れである．夏季から秋季の寒冷に向うと成毛の落毛が遅れて最早対生の成毛の脱落は少なくなり，1毛包に単生する青毛（SY）は極めて稀れとなる．従つて成毛，青毛の対生毛（PO. Y.）から成毛，成毛の対生毛（PO. O.）となり，成毛2，発育毛1の3連生（TO. O. Y., TO. O. C.）の真毛束となる．但し成毛3（TO. O. O.）の連生毛はない．このように後継毛の発生はよどみなく進み，成毛の落毛が停止しているので寒冷時の被毛の量は増している．

このように漸次毛世代を重ねてゆくので11月から毛量は増加して1月→3月が最も多量である．これを福山地方の各月別平均気温と対照すると，摂氏数度から 10°C 前後の11月→3月の5ケ月間にあたつている（第46, 47, 48図）．

この地方では前述のようにプールの換水も稀れで水温は気温の変化にまかせて放置している．これを春秋2季換毛型の動物の場合は毛皮価値のある冬毛の最良期が12月，1月の約2ケ月であつて，換毛の調節の不可能なのに較べて，Nutria は交換毛が季節的に質の上で差異なく（次の寒暖2季の被毛繊度の比較）幅の広い毛皮の利用期間を示している．年間を通じてプールの水を清冷に保つてゆけばさらに毛皮として利用期間は広くなることがうかがえる．

**2. 寒暖2季の被毛繊度の比較**

更に筆者は農家に飼育中の成獣4頭，牝牡2頭宛を選び寒冷季（1月28日），温暖季（5月30日）の2季を選定して採毛し次のような調査結果をえた．即ち牝牡性別の間に被毛の差異はないものとして，この寒暖2季の分岐下毛＋下毛を比較解明することにした．

## 寒暖2の被毛繊度の比較

第97表 (分岐下毛＋下毛の場合)

| 部位 | 動物記号 Animal No. | 寒(A) 暖(B) | 柄幅の相加平均 $\bar{x}$ (μ) | 偏差平方和 $S(x-\bar{x})^2$ | 自由度 N | 分散 $S^2$ | 標準偏差 S (μ) | 変異係数 $\frac{s}{\bar{x}}\times 100$ % |
|---|---|---|---|---|---|---|---|---|
| 背 | NC. 9.4 K♂ | A | 13.76 | 2697.46 | 706 | 3.8262 | 1.96 | 14.24 |
| | | B | 13.36 | 2284.99 | 756 | 3.0225 | 1.74 | 13.02 |
| 背 | ND. 7.9 K♂ | A | 12.56 | 1059.47 | 620 | 1.7088 | 1.31 | 10.42 |
| | | B | 12.50 | 1040.23 | 505 | 2.0599 | 1.44 | 11.52 |
| 背 | NE. 5.3 K♀ | A | 11.81 | 1224.72 | 579 | 1.9697 | 1.40 | 11.85 |
| | | B | 12.04 | 1627.35 | 622 | 2.6163 | 1.62 | 13.45 |
| 背 | NF. 5.6 K♀ | A | 12.01 | 1403.59 | 571 | 2.4581 | 1.57 | 13.07 |
| | | B | 11.97 | 1295.83 | 579 | 2.2381 | 1.37 | 11.45 |
| 腹 | NC. 9.4 K♂ | A | 10.23 | 593.28 | 404 | 1.4685 | 1.21 | 11.83 |
| | | B | 10.35 | 516.56 | 462 | 1.1181 | 1.06 | 10.24 |
| 腹 | ND. 7.9 K♂ | A | 11.41 | 920.35 | 506 | 1.8189 | 1.35 | 11.83 |
| | | B | 10.74 | 825.43 | 717 | 1.1512 | 1.07 | 10.74 |
| 腹 | NE. 5.3 K♀ | A | 10.70 | 1603.11 | 514 | 3.1189 | 1.76 | 16.44 |
| | | B | 10.78 | 1261.01 | 636 | 1.9827 | 1.41 | 13.07 |
| 腹 | NF. 5.6 K♀ | A | 10.46 | 557.74 | 434 | 1.2851 | 1.13 | 10.80 |
| | | B | 10.42 | 1022.16 | 828 | 1.2345 | 1.11 | 10.65 |

第98表 (下毛の場合)

| 部位 | 動物記号 Animal No. | 寒(A) 暖(B) | 柄幅の相加平均 x (μ) | 偏差平方和 $S(x-\bar{x})^2$ | 自由度 N | 分散 $S^2$ | 標準偏差 S (μ) | 変異係数 $\frac{s}{\bar{x}}\times 100$ % |
|---|---|---|---|---|---|---|---|---|
| 背 | NC. 9.4 K♂ | A | 13.51 | 2230.66 | 668 | 3.3393 | 1.83 | 13.54 |
| | | B | 13.25 | 1739.10 | 744 | 2.3375 | 1.53 | 11.54 |
| 背 | ND. 7.9 K♂ | A | 12.30 | 578.51 | 568 | 1.0185 | 1.01 | 8.21 |
| | | B | 12.45 | 1097.40 | 499 | 2.1992 | 1.48 | 11.89 |
| 背 | NE. 5.3 K♀ | A | 11.62 | 845.49 | 550 | 1.5372 | 1.24 | 10.67 |
| | | B | 11.80 | 910.38 | 566 | 1.6085 | 1.29 | 10.93 |
| 背 | NF. 5.6 K♀ | A | 11.74 | 834.73 | 533 | 1.5660 | 1.25 | 10.64 |
| | | B | 11.70 | 761.39 | 537 | 1.4179 | 1.19 | 10.17 |
| 腹 | NC. 9.4 K♂ | A | 10.23 | 593.28 | 504 | 1.4685 | 1.21 | 11.83 |
| | | B | 10.33 | 409.31 | 461 | 0.8878 | 0.94 | 9.10 |
| 腹 | ND. 7.9 K♂ | A | 11.40 | 864.17 | 505 | 1.7112 | 1.31 | 11.49 |
| | | B | 10.74 | 825.43 | 717 | 1.1512 | 1.07 | 10.74 |
| 腹 | NE. 5.3 K♀ | A | 10.55 | 528.41 | 505 | 1.0464 | 1.02 | 9.66 |
| | | B | 10.78 | 1261.01 | 636 | 1.9827 | 1.41 | 13.07 |
| 腹 | NF. 5.6 K♀ | A | 10.42 | 396.60 | 431 | 0.9201 | 0.90 | 8.64 |
| | | B | 10.38 | 823.80 | 824 | 0.9998 | 1.00 | 9.63 |

第 99 表 分岐下毛＋下毛の場合

| 温暖季 | | 寒冷季 | |
|---|---|---|---|
| 動物記号 | 柄幅平均値 (μ) | 動物記号 | 柄幅平均値 (μ) |
| N C 背毛 | 13.36 | N C 背毛 | 13.76 |
| N D 〃 | 12.50 | N D 〃 | 12.56 |
| N E 〃 | 12.04 | N E 〃 | 11.81 |
| N F 〃 | 12.01 | N F 〃 | 12.01 |
| 平均 | 12.47 | 平均 | 12.54 |

$N\bar{x}^2=155.5009\times4=622.0036$

$x_i^2$ { 178.4896, 156.2500, 144.9616, 143.2809

$\Sigma x_i^2=622.9821$

$\Sigma x_i^2-N\bar{x}^2=0.9785$

$N\bar{x}^2=157.2516\times4=629.0064$

$x_i^2$ { 189.3376, 157.7536, 139.4761, 144.2401

$\Sigma x_i^2=630.8074$

$\Sigma x_i^2-N\bar{x}=1.801$

| 動物記号 | 柄幅平均値 (μ) | 動物記号 | 柄幅平均値 (μ) |
|---|---|---|---|
| N C 腹毛 | 10.35 | N C 腹毛 | 10.23 |
| N D 〃 | 10.74 | N D 〃 | 11.41 |
| N E 〃 | 10.78 | N E 〃 | 10.70 |
| N F 〃 | 10.42 | N F 〃 | 10.46 |
| 平均 | 10.57 | 平均 | 10.70 |

$N\bar{x}^2=111.7249\times4=446.8996$

$x_i^2$ { 107.1225, 115.3476, 116.2084, 108.5764

$\Sigma x^2=447.2549$

$\Sigma x_i^2-N\bar{x}^2=0.3553$

$N\bar{x}^2=114.49\times4=457.9600$

$x_i^2$ { 104.6529, 114.4900, 130.1881, 109.4116

$\Sigma x_i^2=458.7426$

$\Sigma x_i^2-N\bar{x}^2=0.7826$

(1) 寒冷季背毛　温暖季背毛　(2) 寒冷季腹毛　温暖季腹毛

(3) 温暖季背毛　温暖季腹毛　(4) 寒冷季背毛　寒冷季腹毛

(1) 寒冷季背毛と温暖季背毛の比較

イ. 仮説　$\sigma_1^2=\sigma_2^2=SP^2$, $M_1=M_2$

ロ. 有意水準：$a=0.05$

ハ. 総計量　$$t=\frac{\bar{X}_1-\bar{X}_2}{SP\sqrt{\frac{1}{N_1}+\frac{1}{N_2}}}$$

2つの母集団は同一平均，同一分散の正規分布とすれば，自由度が $N_1+N_2-2=4+4-2=6$ の t 分布となる，

ニ．棄却域は表により $t>2.45$

ホ．寒冷季　$\bar{X}_1=12.54\,\mu$，　$\Sigma X_{1i}^2-N_1\bar{X}_1^2=1.801\,\mu$

温暖季　$\bar{X}_2=12.47\,\mu$，　$\Sigma X_{2i}^2-N_2\bar{X}_2^2=0.9785\,\mu$

$$\therefore\quad SP^2=\frac{0.9785+1.801}{6}=\frac{1.1586}{6}=0.1931$$

$$SP=0.4394$$

$$\therefore\quad t=\frac{12.54-12.47}{0.4394\times0.7071}=\frac{0.07}{0.3107}=0.225$$

ヘ．これは棄却域にないから仮説は棄却出来ない．両者に有意な差があると言えない．よつて背毛（分岐下毛＋下毛）は寒暖の差異は認められない．

(2) 寒冷季腹毛と温暖季腹毛の比較

寒冷季腹毛　$\bar{X}_1=10.70\,\mu$，　$\Sigma X_{1i}^2-N_1\bar{X}_1^2=0.7826$

温暖季腹毛　$\bar{X}_2=10.57\,\mu$，　$\Sigma X_{2i}^2-N_2\bar{X}_2^2=0.3553$

$$\therefore\quad SP^2=\frac{0.7826+0.3553}{6}=0.1896$$

$$SP=0.4354$$

$$\therefore\quad t=\frac{10.70-10.57}{0.4354\times0.7071}=0.422$$

これは棄却域にないから仮説は棄却出来ない．両者に有意な差があると言えない．よつて腹毛（分岐下毛＋下毛）は寒暖の差異は認められない．

(3) 温暖季背毛と温暖季腹毛の比較

温暖季背毛　$\bar{X}_1=12.47\,\mu$，　$\Sigma X_{1i}^2-N_1\bar{X}_1^2=0.9785$

温暖季腹毛　$\bar{X}_2=10.57\,\mu$，　$\Sigma X_{2i}^2-N_2\bar{X}_2^2=0.3553$

$$\therefore\quad SP^2=\frac{0.9785+0.3553}{6}=0.2223$$

$$SP=0.4715$$

$$\therefore\quad t=\frac{12.47-10.57}{0.4715\times0.7071}=5.7$$

これは棄却域にあり，仮説は棄却せられる．両者は非常に有意な差異が認められる．よつて温暖季において背毛と腹毛の差異が認められる．

(4) 寒冷季背毛と寒冷季腹毛の比較

寒冷季背毛　$\bar{X}_1=12.54\,\mu$，　$\Sigma X_{1i}^2-N_1\bar{X}_1^2=1.801\,\mu$

寒冷季腹毛　$\bar{X}_2=10.70\,\mu$，　$\Sigma X_{2i}^2-N_2\bar{X}_2^2=0.7826\,\mu$

$$\therefore\quad SP^2=\frac{1.801+0.7826}{6}=0.4306$$

$$SP=0.6562$$

$$\therefore\quad t=\frac{12.54-10.70}{0.6562\times0.7071}=4.07$$

両者は有意な差異が認められる．よつて寒冷季において背毛と腹毛の差異は認められる．

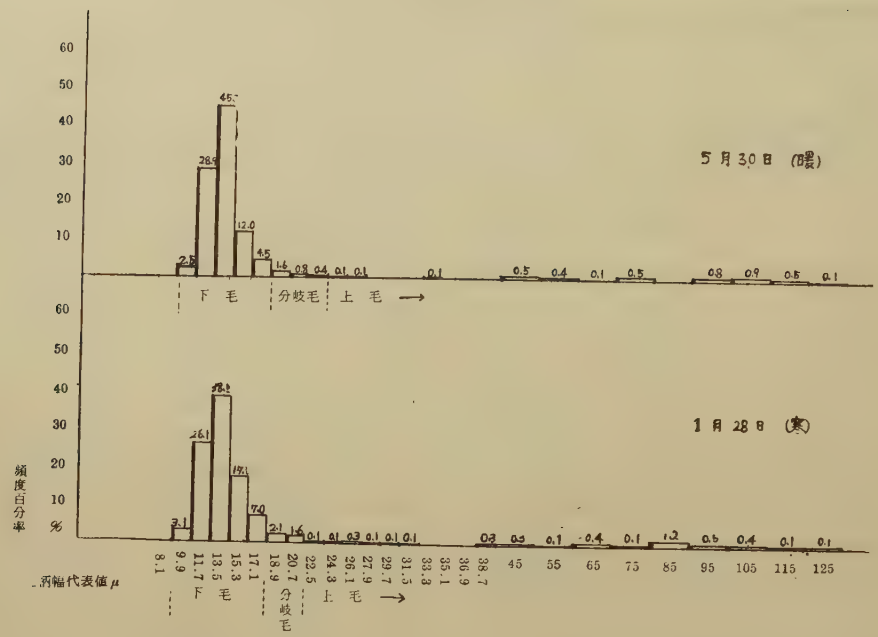
第 100 表 成獣被毛の度数分布表
(動物記号 NC, ND, 背毛)

| 動物記号 → | | NC 背毛 | | | | | | | | ND 背毛 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 柄 | 幅 | 寒 | | | | 暖 | | | | 寒 | | | | 暖 | | | |
| 1.8μ 間隔代表値 (A) μ | 10μ 間隔代表値 (B) μ | No. | | % | | No. | | % | | No. | | % | | No. | | % | |
| | | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| 9.9 | | 23 | | 3.1 | | 20 | | 2.5 | | 26 | | 3.8 | | 39 | | 7.0 | |
| 11.7 | | 197 | | 26.1 | | 231 | | 28.9 | | 327 | | 48.2 | | 253 | | 45.2 | |
| 13.5 | | 290 | | 38.5 | | 362 | | 45.3 | | 216 | | 31.8 | | 170 | | 30.4 | |
| 15.3 | | 129 | | 17.1 | | 96 | | 12.0 | | 54 | | 7.9 | | 38 | | 6.8 | |
| 17.1 | | 53 | | 7.0 | | 36 | | 4.5 | | 13 | | 1.9 | | 17 | | 3.0 | |
| 18.9 | 15 | 16 | 708 | 2.1 | 93.9 | 13 | 758 | 1.6 | 94.8 | 8 | 644 | 1.2 | 94.8 | 3 | 520 | 0.5 | 92.9 |
| 20.7 | | 12 | | 1.6 | | 6 | | 0.8 | | 5 | | 0.7 | | 5 | | 0.9 | |
| 22.5 | | 1 | | 0.1 | | 3 | | 0.4 | | 5 | | 0.7 | | 0 | | 0 | |
| 24.3 | | 1 | | 0.1 | | 1 | | 0.1 | | 1 | | 0.1 | | 1 | | 0.2 | |
| 26.1 | | 2 | | 0.3 | | 1 | | 0.1 | | 1 | | 0.1 | | 1 | | 0.2 | |
| 27.9 | 25 | 1 | 17 | 0.1 | 2.3 | | 11 | | 1.4 | 2 | 14 | 0.3 | 2.1 | 2 | 9 | 0.4 | 1.6 |
| 29.7 | | 1 | | 0.1 | | | | | | 0 | | 0 | | 2 | | 0.4 | |
| 31.5 | | 1 | | 0.1 | | | | | | 0 | | 0 | | 2 | | 0.4 | |
| 33.3 | | 0 | | 0 | | 1 | | 0.1 | | 0 | | 0 | | 2 | | 0.4 | |
| 35.1 | | 0 | | 0 | | | | | | 2 | | 0.3 | | 1 | | 0.2 | |
| 36.9 | | 0 | | 0 | | | | | | 0 | | | | 0 | | 0 | |
| 38.7 | 35 | 2 | 4 | 0.3 | 0.5 | | 1 | | 0.1 | 0 | 2 | | 0.3 | 0 | 7 | 0 | 1.3 |
| | 45 | | 2 | | 0.3 | | 4 | | 0.5 | | 4 | | 0.6 | | 1 | | 0.2 |
| | 55 | | 1 | | 0.1 | | 3 | | 0.4 | | 1 | | 0.1 | | 4 | | 0.7 |
| | 65 | | 3 | | 0.4 | | 1 | | 0.1 | | 2 | | 0.3 | | 3 | | 0.5 |
| | 75 | | 1 | | 0.1 | | 4 | | 0.5 | | 1 | | 0.1 | | 4 | | 0.7 |
| | 85 | | 9 | | 1.2 | | | | | | 2 | | 0.3 | | 4 | | 0.7 |
| | 95 | | 4 | | 0.5 | | 6 | | 0.8 | | 6 | | 0.9 | | 2 | | 0.4 |
| | 105 | | 3 | | 0.4 | | 7 | | 0.9 | | 1 | | 0.1 | | 4 | | 0.7 |
| | 115 | | 1 | | 0.1 | | 4 | | 0.5 | | 2 | | 0.3 | | 1 | | 0.2 |
| | 125 | | 1 | | 0.1 | | 1 | | 0.1 | | | | | | 1 | | 0.2 |
| | | | 754 | | 99.9 | | 800 | | 100.1 | | 679 | | 99.9 | | 560 | | 100.1 |

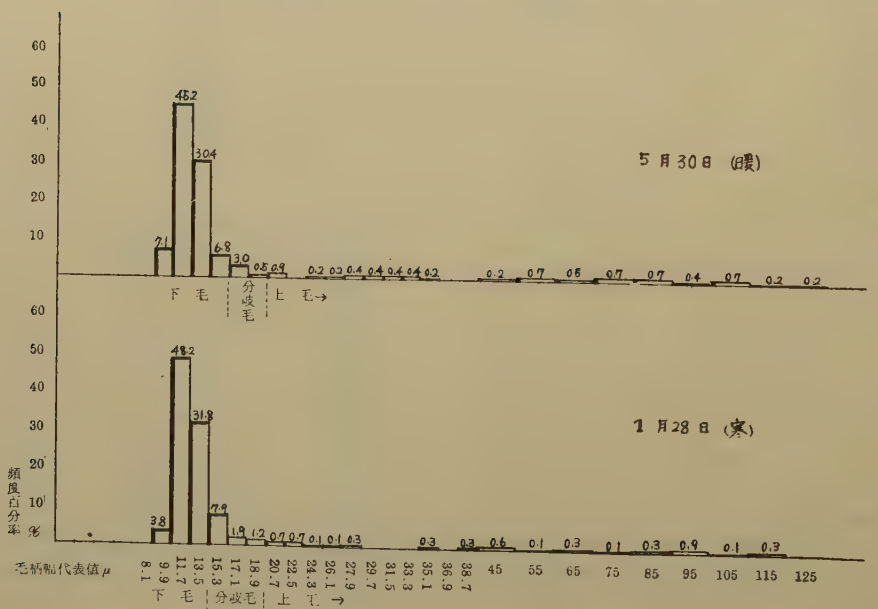
第101表　成獣被毛の度数分布表
（動物記号 NE, NF, 背毛）

| 動物記号 → | | NE 背毛 | | | | | | | | NF 背毛 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 柄 | 幅 | 寒 | | | | 暖 | | | | 寒 | | | | 暖 | | | |
| 1.8μ間隔代表値 (A) μ | 10μ間隔代表値 (B) μ | No. | | % | | No. | | % | | No. | | % | | No. | | % | |
| | | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| 9.9 | | 143 | | 21.8 | | 139 | | 19.5 | | 122 | | 19.1 | | 117 | | 17.8 | |
| 11.7 | | 289 | | 44.1 | | 286 | | 40.2 | | 276 | | 43.1 | | 303 | | 46.0 | |
| 13.5 | | 119 | | 18.2 | | 142 | | 20.0 | | 136 | | 21.3 | | 118 | | 17.9 | |
| 15.3 | | 45 | | 6.9 | | 62 | | 8.7 | | 45 | | 7.0 | | 40 | | 6.1 | |
| 17.1 | | 18 | | 2.7 | | 27 | | 3.8 | | 18 | | 2.8 | | 23 | | 3.5 | |
| 18.9 | 15 | 9 | 623 | 1.4 | 95.0 | 13 | 669 | 1.8 | 94.1 | 6 | 603 | 0.9 | 94.2 | 11 | 612 | 1.7 | 92.9 |
| 20.7 | | 5 | | 0.8 | | 7 | | 1.0 | | 3 | | 0.5 | | 10 | | 1.5 | |
| 22.5 | | 5 | | 0.8 | | 5 | | 0.7 | | 6 | | 0.9 | | 2 | | 0.3 | |
| 24.3 | | 3 | | 0.5 | | 3 | | 0.3 | | 2 | | 0.3 | | 2 | | 0.3 | |
| 26.1 | | 1 | | 0.2 | | 4 | | 0.6 | | 3 | | 0.5 | | 2 | | 0.3 | |
| 27.9 | 25 | 3 | 17 | 0.5 | 2.6 | 1 | 20 | 0.1 | 2.8 | 1 | 15 | 0.2 | 2.3 | 2 | 18 | 0.3 | 2.7 |
| 29.7 | | 1 | | 0.2 | | 2 | | 0.2 | | 2 | | 0.3 | | 1 | | 0.2 | |
| 31.5 | | 0 | | 0 | | 0 | | 0 | | 1 | | 0.2 | | 0 | | 0 | |
| 33.3 | | 0 | | 0 | | 1 | | 0.1 | | 1 | | 0.2 | | 0 | | 0 | |
| 35.1 | | 1 | | 0.2 | | 1 | | 0.1 | | 0 | | 0 | | 1 | | 0.2 | |
| 36.9 | | 1 | | 0.2 | | 0 | | 0 | | 1 | | 0.2 | | 0 | | 0 | |
| 38.7 | 35 | 1 | 4 | 0.2 | 0.6 | | 4 | | 0.6 | 2 | 7 | 0.3 | 1.1 | 2 | 4 | 0.3 | 0.6 |
| | 45 | | 6 | | 0.9 | | 1 | | 0.1 | | 3 | | 0.5 | | 3 | | 0.5 |
| | 55 | | 0 | | | | 2 | | 0.2 | | 3 | | 0.5 | | 0 | | 0 |
| | 65 | | 3 | | 0.5 | | 3 | | 0.3 | | 6 | | 0.9 | | 2 | | 0.3 |
| | 75 | | 0 | | | | 3 | | 0.3 | | 0 | | 0 | | 5 | | 0.8 |
| | 85 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | 0 | | 1 | | 0.2 |
| | 95 | | 0 | | | | 1 | | 0.1 | | 3 | | 0.5 | | 6 | | 0.9 |
| | 105 | | 1 | | 0.2 | | 1 | | 0.1 | | | | | | 3 | | 0.5 |
| | 115 | | 1 | | 0.2 | | 1 | | 0.1 | | | | | | 1 | | 0.2 |
| | 125 | | | | | | 4 | | 0.6 | | | | | | 4 | | 0.6 |
| | 135 | | | | | | 1 | | 0.1 | | | | | | | | |
| | | | 656 | | 100.2 | | 711 | | 99.4 | | 640 | | 100 | | 659 | | 100.2 |

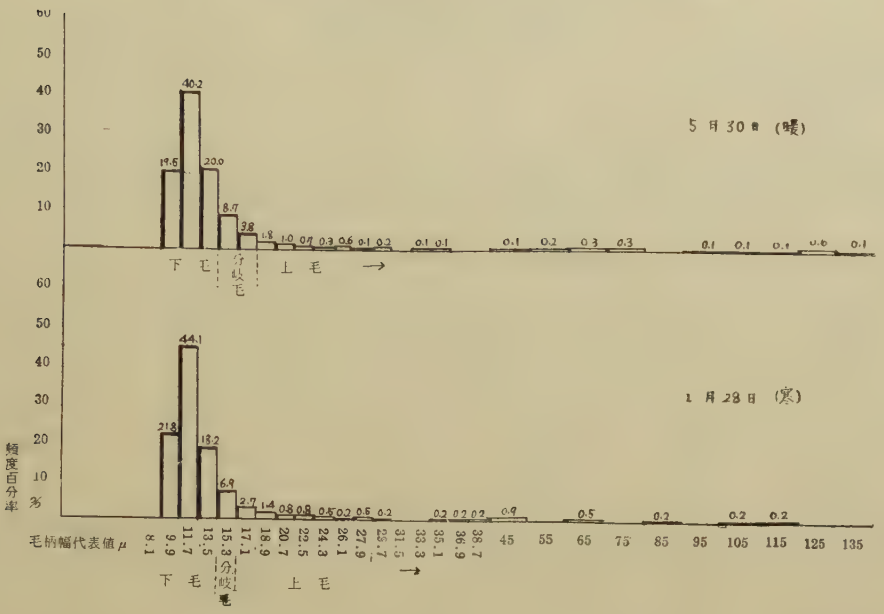
第 49 図　寒暖 2 季の被毛比較図♂（動物記号 NC 背毛）



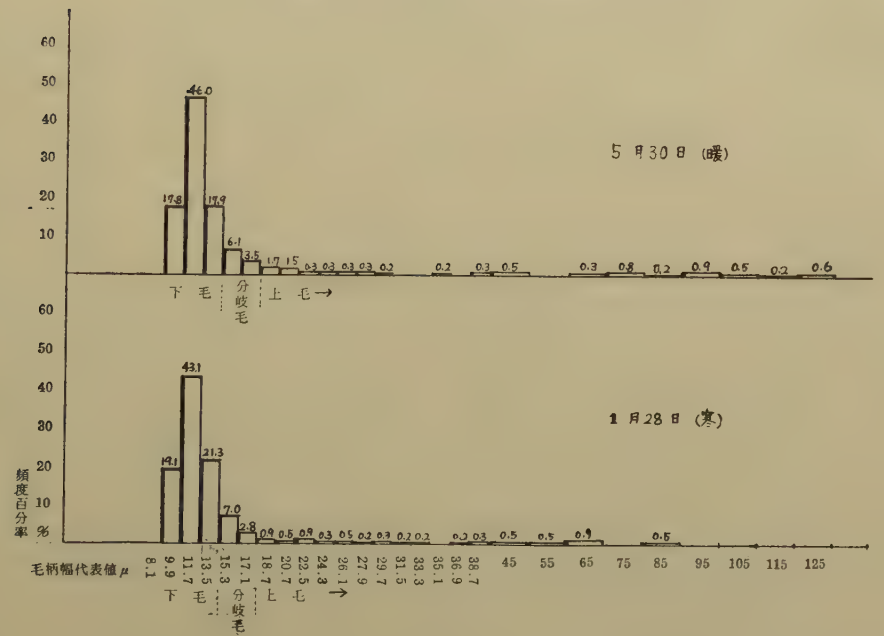

第 50 図　寒暖 2 季の被毛比較図　♂動物記号 ND 背毛



第 51 図　寒暖 2 季の被毛比較図　♀動物記号 NE 背毛




第 52 図　寒暖 2 季の被毛比較図　♀動物記号 NF 背毛



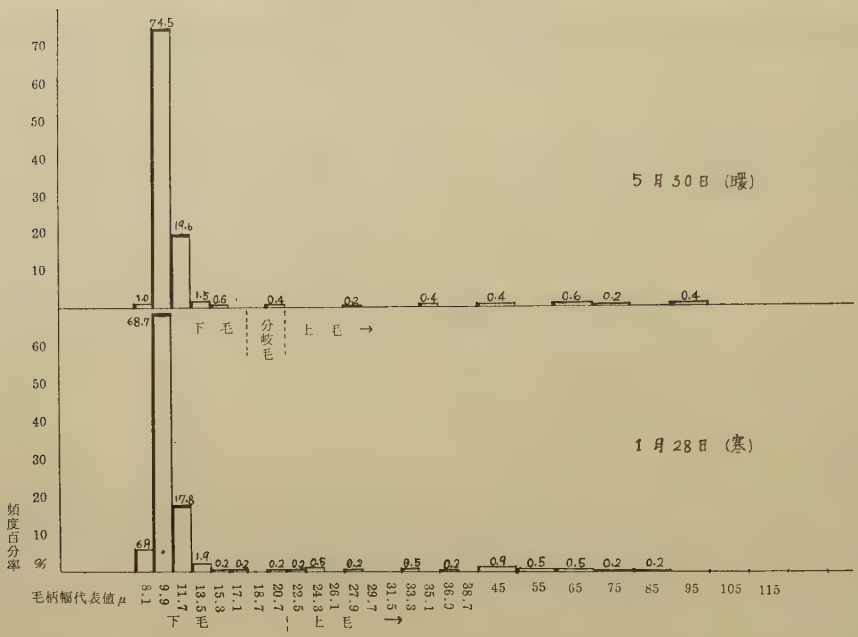
第102表 成獣被毛の度数分布表
(動物記号 NC, ND, 腹毛)

| 動物記号 → | | NC 腹毛 | | | | | | | | ND 腹毛 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 柄 | 幅 | 寒 | | | | 暖 | | | | 寒 | | | | 暖 | | | |
| | | No. | | % | | No. | | % | | No. | | % | | No. | | % | |
| 1.8μ間隔代表値 (A) μ | 10μ間隔代表値 (B) μ | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| 8.1 | 5 | 29 | 29 | 6.9 | 6.9 | 5 | 5 | 1.0 | 1.0 | | | | | | | | |
| 9.9 | | 290 | | 68.7 | | 354 | | 74.5 | | 168 | | 32.1 | | 405 | | 55.5 | |
| 11.7 | | 75 | | 17.8 | | 93 | | 19.6 | | 267 | | 51.1 | | 298 | | 40.8 | |
| 13.5 | | 8 | | 1.9 | | 7 | | 1.5 | | 62 | | 11.9 | | 11 | | 1.5 | |
| 15.3 | | 1 | | 0.2 | | 3 | | 0.6 | | 6 | | 1.1 | | 2 | | 0.3 | |
| 17.1 | | 1 | | 0.2 | | 0 | | 0 | | 3 | | 0.6 | | 1 | | 0.1 | |
| 18.9 | 15 | 0 | 375 | 0 | 88.9 | 0 | 457 | 0 | 96.2 | 2 | 508 | 0.4 | 97.1 | 0 | 717 | 0 | 98.2 |
| 20.7 | | 1 | | 0.2 | | 2 | | 0.4 | | 0 | | | | 1 | | 0.1 | |
| 22.5 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | | | 0 | | 0 | |
| 24.3 | | 2 | | 0.5 | | 0 | | 0 | | 0 | | | | 0 | | 0 | |
| 26.1 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | 1 | | 0.1 | |
| 27.9 | 25 | 1 | 5 | 0.2 | 1.2 | 1 | 3 | 0.2 | 0.6 | 0 | 0 | | 0 | 0 | 2 | 0 | 0.3 |
| 29.7 | | 0 | | 0 | | 0 | | 0 | | 1 | | 0.2 | | 1 | | 0.1 | |
| 31.5 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | 1 | | 0.1 | |
| 33.3 | | 2 | | 0.5 | | 0 | | 0 | | 0 | | | | 0 | | 0 | |
| 35.1 | | 0 | | 0 | | 2 | | 0.4 | | 0 | | | | 0 | | 0 | |
| 36.9 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | | | 0 | | 0 | |
| 38.7 | 35 | 0 | 3 | 0 | 0.7 | 0 | 2 | 0 | 0.4 | 0 | 1 | | 0.2 | 1 | 3 | 0.1 | 0.4 |
| | 45 | | 4 | | 0.9 | | 2 | | 0.4 | | 1 | | 0.2 | | 0 | | 0 |
| | 55 | | 2 | | 0.5 | | 0 | | 0 | | 1 | | 0.2 | | 3 | | 0.4 |
| | 65 | | 2 | | 0.5 | | 3 | | 0.6 | | 2 | | 0.4 | | 1 | | 0.1 |
| | 75 | | 1 | | 0.2 | | 1 | | 0.2 | | 0 | | 0 | | 2 | | 0.3 |
| | 85 | | 1 | | 0.2 | | 0 | | 0 | | 2 | | 0.4 | | 2 | | 0.3 |
| | 95 | | | | | | 2 | | 0.4 | | 4 | | 0.8 | | | | |
| | 105 | | | | | | | | | | 3 | | 0.6 | | | | |
| | 115 | | | | | | | | | | 1 | | 0.2 | | | | |
| | | | 422 | | 100 | | 475 | | 99.8 | | 523 | | 100.1 | | 730 | | 100 |

第103表　成獣被毛の度数分布表
（動物記号 NE, NF, 腹毛）

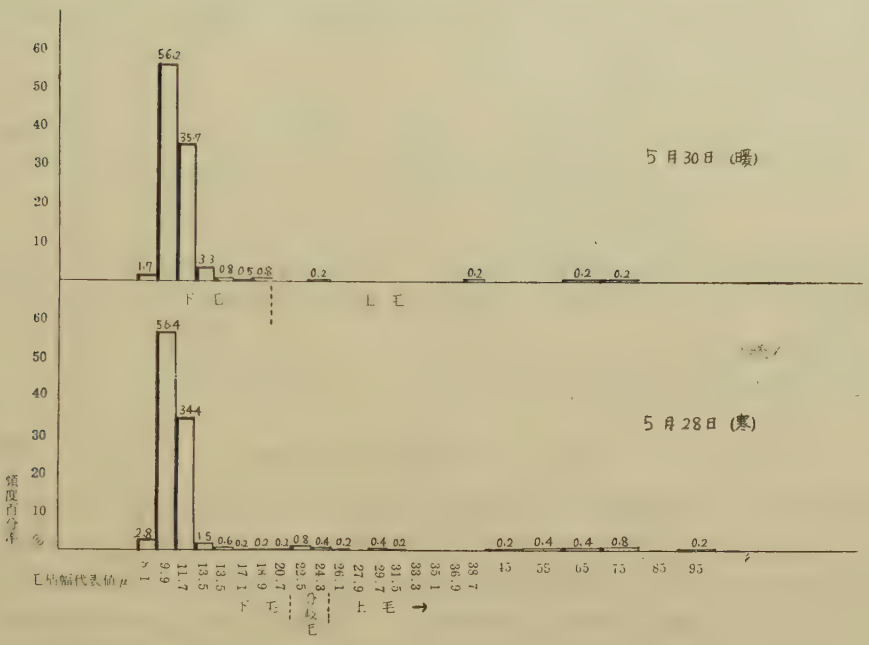
| 動物記号→ | | NE 腹毛 | | | | | | | | NF 腹毛 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 柄 | 幅 | 寒 | | | | 暖 | | | | 寒 | | | | 暖 | | | |
| 1.8μ間隔代表値 (A) μ | 10μ間隔代表値 (B) μ | No. | | % | | No. | | % | | No. | | % | | No. | | % | |
| | | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| 8.1 | 5 | 15 | 15 | 2.8 | 2.8 | 11 | 11 | 1.7 | 1.7 | 13 | 13 | 2.9 | 2.9 | 31 | 31 | 3.7 | 3.7 |
| 9.9 | | 300 | | 56.4 | | 362 | | 56.2 | | 286 | | 64.4 | | 556 | | 66.5 | |
| 11.7 | | 183 | | 34.4 | | 230 | | 35.7 | | 129 | | 29.1 | | 223 | | 26.7 | |
| 13.5 | | 8 | | 1.5 | | 21 | | 3.3 | | 4 | | 0.9 | | 15 | | 1.8 | |
| 15.3 | | 3 | | 0.6 | | 5 | | 0.8 | | 1 | | 0.2 | | 3 | | 0.4 | |
| 17.1 | | 1 | | 0.2 | | 3 | | 0.5 | | 1 | | 0.2 | | 1 | | 0.1 | |
| 18.9 | 15 | 1 | 496 | 0.2 | 93.2 | 5 | 626 | 0.8 | 97.2 | 3 | 424 | 0.7 | 95.5 | 0 | 798 | 0 | 95.5 |
| 20.7 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | 0 | | 1 | | 0.1 | |
| 22.5 | | 4 | | 0.8 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| 24.3 | | 2 | | 0.4 | | 1 | | 0.2 | | 0 | | 0 | | 1 | | 0.1 | |
| 26.1 | | 1 | | 0.2 | | 0 | | 0 | | 1 | | 0.2 | | 0 | | 0 | |
| 27.9 | 25 | 0 | 8 | 0 | 1.5 | 0 | 1 | 0 | 0.2 | 0 | 1 | 0 | 0.2 | 1 | 3 | 0.1 | 0.4 |
| 29.7 | | 2 | | 0.4 | | 0 | | 0 | | 1 | | 0.2 | | | | | |
| 31.5 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | 0 | | | | | |
| 33.3 | | | | | | 0 | | 0 | | 0 | | 0 | | | | | |
| 35.1 | | | | | | 0 | | 0 | | 1 | | 0.2 | | | | | |
| 36.9 | | | | | | 0 | | 0 | | | | | | | | | |
| 38.7 | 35 | | 3 | | 0.6 | 1 | 1 | 0.2 | 0.2 | | 2 | | 0.5 | | | | |
| | 45 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | 0 | | 1 | | 0.1 |
| | 55 | | 2 | | 0.4 | | 0 | | 0 | | 1 | | 0.2 | | 1 | | 0.1 |
| | 65 | | 2 | | 0.4 | | 1 | | 0.2 | | 0 | | 0 | | 0 | | 0 |
| | 75 | | 4 | | 0.8 | | 1 | | 0.2 | | 1 | | 0.2 | | 0 | | 0 |
| | 85 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 |
| | 95 | | 1 | | 0.2 | | 3 | | 0.5 | | 1 | | 0.2 | | 2 | | 0.2 |
| | 105 | | | | | | | | | | 1 | | 0.2 | | | | |
| | | | 532 | | 100.2 | | 644 | | 100.2 | | 444 | | 99.9 | | 836 | | 100 |

第 53 図　寒暖 2 季の被毛比較図　♂動物記号 NC 腹毛

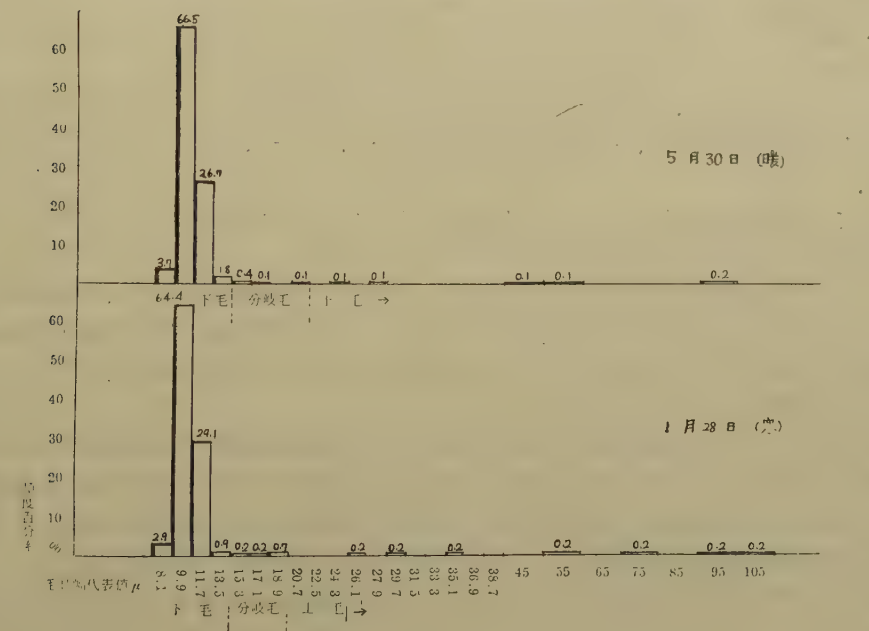



第 54 図　寒暖 2 季の被毛比較図　♂動物記号ND 腹毛

第 55 図　寒暖 2 季の被毛比較図　♀動物記号 NE 腹毛




第 56 図　寒暖 2 季の被毛比較図　♀動物記号 NF 腹毛



考察，前述の毛量の年間変遷で月別調査によれば同じ毛包から生じた温暖季の被毛は寒冷季に向かつて棍状毛(成毛)となつて対生毛，連生毛として同じ毛管(hair canal)に真毛束を形成して留つているので寒暖2季の被毛が混生しており，とくに寒冷季に生じたもののみを測定することは出来ない，又温暖季（6月，7月）暑熱季の被毛には寒冷季に発生したものは全くないが，比較に使用した採毛資料は5月30日の採毛であるので寒冷季の被毛の混入が考えられる．以上のように厳密な意味で言えば完全な寒冷季．温暖季発生の被毛のみの比較は不可能であるが，その傾向についてはうかがえる訳けである。ヒストグラムで比較すればその百分率の頻度分布はほぼ同形を保つている．又下毛の繊度を表わす柄幅の平均値に大きな差異は認められないので，上述のように統計量を求めて両季の背毛，腹毛について比較を試みた結果背毛，腹毛ともにその繊度の差異はないようである．又両季とも背毛と腹毛との差異は常に明らかに現われている．

## IX. 結 論 及 び 摘 要

1. Nutria の生毛の毛原基は3ケ月胎児で略整つている。既に背毛被毛の3原毛群は鞘毛まで発育している．頭，背，腹，胸部の順序に発生しており，胸部は未だ色素のない毛芽である．従つて3ケ月胎児は頭部，背部が薄墨色の裸形である．

2. 出生時の幼獣は既に全身が生毛で被われ，その被毛の1部は大型毛で毛長約$\frac{1}{4}$，小型毛（分岐毛以下）は約$\frac{1}{2}$に発育しており，生後数時間の後には游浴時の保温に役立つている．この際3原毛は幼毛，青毛であるが副側毛の多くは毛芽と鞘毛である．未だ$\frac{1}{2}$次副側毛の発生の兆はない．

3. 生後1ケ月幼獣までは生毛の完成期で全生毛は成毛と青毛で，成毛の少数は後継毛の毛芽を伴なつているが，2次副側毛の幼毛の発生は極めて稀れである．

4. 生毛群は中央毛，側毛の3原毛を中間に左右に副側毛を配して最多数15毛，最少数3毛で1毛群が構成せられ，一般に生毛群は15毛型→7毛型→3毛型とその中間型で成立している．

5. 2次副側毛の発生は生後2ケ月頃最も盛んで8ケ月までその発生が確認されており，12ケ月後には発生はない．

6. これら数個の2次副側毛包が生毛包を中心にして毛束を形成するが，この際大型毛群では3原毛は大型上毛で毛包は独立する場合が多く，毛束の形成は生毛の副側毛を中心に2次副側毛で成立する．7毛型では中央毛をのぞく生毛包を中心に毛束が成立し，3毛型では3生毛包を中心に3毛束毛群となる．

以上のように上毛の独立毛包は単生毛包として又は後継毛を伴なう対生毛束，極めて稀れに3連生毛束として，その他は後継毛と隣接の毛包を合せた偽毛束として略々生毛群時代の毛包数に等しい毛束数で毛群を完成する．成獣では毛群の密度は幼獣の約4％にすぎないが毛量の密度は約50％である．

7. 凡て毛包の発生は所謂分芽によらず，新毛包に隣接して，個々に胚芽層から陥入して発生する．

8. 後継毛の先端を包んだ上皮性組織は常に成毛の棍球が埋づまつている被膜の外部である上皮性組織に附着し，これに沿つて上昇して．皮脂腺位で同じ毛管内に合流して体表に現

われている．とくに第1上毛，第2上毛の大型上毛の幼毛は成毛に背負われたような形で上方に伸び出して成毛を保護しているようである．

9. 後継毛の発生は不断に寒冷，温暖の別なく動物の健康が常態である限り同調で繰り返えされている．

10. 上毛，下毛ともに動物の成育期間は換毛毎に漸次郭大しているが，成長後は変化なく，とくに下毛は現状を維持しており，更に性別においても差異を認めない．又四季を通じて変化はない．但し背下毛は常に腹下毛より大であることは明らかである．

11. 第1，第2上毛の大型毛は寒暖の別なくその滞留期間は発生後4.5—5.0ケ月間であるが，第2上毛の小型毛，下毛は温暖季の滞留期間は3.5—4.0ケ月間でやや短く，寒冷季は5.5—6.0ケ月で上毛より長い．

12. 生後12ケ月以後は新毛包の増加はなく，成毛の滞留と後継毛の追補によつて，1毛包から対生毛束，連生毛束（連世代毛束）の真毛束を生じ，これらが合併して偽毛束を形成する．寒冷季においては，1毛包の形成する世代毛束の毛数は3毛が最高で3毛が凡て成毛のことはない．成毛2，発育毛1の組合せである（TGnO, $Gn_{+1}O$, $Gn_{+2}$, Y 又は TGnO, $Gn_{+1}O$, $Gn_{+2}$, C）。

暑熱季においては3連生毛束はなく，凡て成毛と発育毛との対生毛束で（PGnO, $Gn_{+1}Y$ 又は PGnO, $Gn_{+1}$, C），成毛と成毛との対生毛束はない（PGnO, $Gn_{+1}O$）．

従つて寒冷季は温暖季の約2倍の毛量を示している．

13. 下毛の毛髄は気室と髄質と交互に並んだ連鎖状である．毛髄は髄質細胞が一列に連続している場合だけ完全に閉じた個々の気室を形成している．この気室は髄質の核形質が占めていた空間を中心にした空洞であつて従つて気室は細胞内に形成されている．

14. 上毛の毛髄は角化した細胞形質が皮質の孔道壁に略等間隔に附着して互いに周縁から内部に伸びて接着し，対面し，又は交叉してその間にジグザグ通気洞を形成している．

15. 下毛の毛柄の場合は色素顆粒は気室を隔てる髄質に多量に存在しているが，上毛の毛柄では色素は皮質と髄質に平均して分散している．

16. 毛小皮は皮質と髄質とよりは早期に核形質の染着性が消失するので，毛小皮の細胞形質の角化は皮質，髄質より早く，皮質と髄質の核形質の染着性は略同時に失われて，角化は同時のようである．

17. 下毛の捲縮は毛の発育中に毛球の上部において，毛小皮細胞が（特に核形質）皮質細胞の均等な配置を妨げることから起る．

背毛，腹毛ともに定形的な捲縮のある下毛の毛幅の限界は柄幅約13 $\mu$で，単一の毛小皮細胞で毛柄を完全に包囲する下毛の限界は柄幅約20 $\mu$で，毛葉の縄紋型冠状毛小皮の限界は葉幅で約25 $\mu$である．

18. 毛小皮が冠状で単一細胞のときは，髄質細胞も縦に一列の配置をする．

19. 毛柄の横断面で，毛小皮，髄質，皮質細胞が単数から複数に（皮質細胞が1重から2重に）移行する境が下毛と上毛の岐れ目にあたる．

20. Nutria の被毛は次のように分類される．

第104表 Nutria の被毛の分類表

| 分類名称 | | 毛長 cm | 柄幅 μ | 葉幅 μ | 毛量 % |
|---|---|---|---|---|---|
| (1) 第1上毛 | 背毛 | 2.0～8.0 | 15～125 | 20～200 | 5～10 |
| (2) 第2上毛 | 腹毛 | 1.5～5.0 | 15～ 85 | 20～120 | 1～ 5 |
| (3) 分岐毛 分岐上毛 | 背毛 | 2.0～2.5 | 12～ 14 | 12～ 25 | 90～95 |
| (4) 下毛 | 腹毛 | 1.5～2.0 | 10～ 12 | 10～ 20 | 95～99 |

(1) 第1上毛，長大毛で，毛頸部は狭窄せず．黒褐色で明葉帯のないのを原則とするが，毛葉先端（毛芒の下方）が淡色のこともある．

(2) 第2上毛，毛長は第1上毛に及ぶものもある．毛葉は幅広く葉腹の中央に明葉帯があり，毛幹の中央が狭窄して毛頸は明らかに現われ，毛柄の幅は第1上毛に勝る場合も多い．

(3) 分岐毛，明らかに上毛と下毛と分離可能である．分岐上毛は毛柄は分岐下毛に等しいが，毛葉はやや幅広く明葉帯が毛葉腹にあり明らかに上毛的な性格を備えている．

分岐下毛は下毛の大型毛で毛柄は分岐上毛と等しく，又毛柄，毛頸，毛葉等の幹幅の比率は下毛と同様である．分岐毛とは，両者の毛柄の幹幅（柄幅）が同じ級間にあるので，これを呼称する．この級間を分岐域（分岐帯）と呼ぶ．

(4) この分岐域外にある下毛は単に下毛と呼ぶ．

21. Nutria の被毛は生後約12ケ月以後には新らしく毛包の発生を見ない．生後数ケ月後には体表の毛群密度は激減するが，毛包の密度は2次副側毛包の増加で補われているので，成獣の被毛密度は幼獣時代の約半量で留まっている．この生後数ケ月間（とくに寒冷季の場合）が Nutria の生涯を通じて被毛密度は最高で，繊細な下毛を持つた毛皮を生産する．然し乍らとくにこの動物は腹毛の密度が高くて繊細なため毛皮は背割りにされて珍重されるが，幼獣は腹部の表皮組織が薄く脆いので，生後12ケ月以上，体重が約4－5瓩のものから毛皮として利用価値が増して来る．更に春秋2季に更衣する動物の被毛が酷寒季の12月，1月の約2ケ月間のみ新鮮で佳良な冬毛の毛皮を提供するのに較べて，一方 Nutria は年間不断に換毛が行なわれていて，寒冷季はことに成毛の落毛は停止し毛量が増加する．

これは厳冬に限らず晩春においても同様であるので，水陸両棲のこの動物は清冽な湧水，流水を浴槽に導入して管理を怠らねば毛皮として幅の広い利用期間が得られる．

22. なお本論において，従来の文献に対する新説，更に現在まで未知の事柄についての論説を掲げれば次のようである．

(1) 毛群内の毛包の配置を解明したこと．

(2) 下毛の髄質細胞内に生ずるとされた気室を更に細胞核の占めていた空間であるとしたこと．

(3) 羊毛の捲縮（crimps）は化学的に解明されたが，Nutria の下毛の捲縮では細胞の配置に因つて起ること．

(4) 上毛と下毛の分岐域は細胞の配列が単数から複数に移行する経過域であること．

(5) Nutriaの成獣の下毛は牝牡別，年齢別及び季節的な差異がないこと．

(6) Nutria の場合，年間毛の発生量に差異はないが，寒冷季は脱毛が稀れで，温暖季は

1毛包に1—2毛，寒冷季には2—3毛を留めていること．

(7) 春秋2季に明らかに性状の異る被毛が交換する動物の毛皮はその採集可期が限られているのに較べて，年間間断なく換毛する Nutria はその飼育管理によつて更にその毛皮の採集期間を延長しうること．

## X. SUMMARY AND CONCLUSIONS.

1) In the three months old foetus of the nutria, the development of the lanugo hair anlage are almost completed, and the dorsal original trio group are growing to be "Scheiden haare".

The hair anlage developes in the order of caput, dorsum, abdomen and pectus and so the anlage on the pectus is still in hair germ stage being devoid of pigment. Therefore three months foetus is of a dark gray nude figure.

2) At birth a baby animal is covered with lanugo hairs. In about a half number of lanugo hair follicles contained in the hair groups the large-type hairs are growing to about one-fourth of the whole length and the small-type hairs to about a half of the length.

At several hours after birth, they may be served as keeping the body heat in the cold water.

At birth original trio group are still young or the younger hairs and most of the sublateral hairs are in hair germ state "Scheiden haare".

There is no sign of the postnatal sublateral hair growth.

3) During about a month after birth, the lanugo hair groups accomplish their development and most of the lanugo hairs become club hairs while the rest of them remain as young hairs.

A small number of club hairs are accompanied with their successors.

The development of new postnatal sublateral hair follicle occurs rarely.

4) The lanugo hair group is composed of original trio group and sublateral hairs are arranged on both sides of trio group. The largest lanugo hair group has fifteen follicles and the smallest group three of them.

Generally, lanugo hair groups are of "Fifteen hairs type", "Seven hairs type", "Three hairs type", or middle number type.

5) The development of new postnatal sublateral hair follicle is most active at about two months after birth. It is evident that the additional development of new hair follicles is continued until eight month old and ceased before twelve months.

6) The pseudo hair bundle is made of several post sublateral hair follicles concentrating around a lanugo cross-hair follicle or a lanugo under hair follicle. In this occasion the original trio group contained in the large hair group, fifteen lanugo hair type group, are composed of a first over hair and two second over hairs, follicles of which are almost independent from pseudo hair bundles.

In the hair group of the seven lanugo hair type, the group is composed of six pseudo hair bundles and a solitary central hair follicle. In the hair group of the three lanugo hair type, the group is composed of three pseudo hair bundles concentrating around original trio hair follicles.

In such a way above mentioned, every large over hair follicle gives rise to a solitary hair, and then a generation of coupled hair bundle, or rarely a generation of trio hair bundle. Other small over hair follicle, cross hair follicle, or under hair follicle passes a solitary hair and the generation of true bundle occurs like the large over hair follicles. These solitary hairs and true bundles are combined together to make several pseudo hair bundles.

The hair groups of the adult pelage are composed of the above mentioned hair bundles which are about the same number as the follicles of lanugo hair groups.

The hair group density of the adult pelage is only about four percent of the juvenal pelage, but the hair density of the adult is about a half of the juvenal pelage.

7) It seems highly probable that the development of all follicles is not brought about by the process of proliferation, but develops and migrates down from the stratum germinativum.

8) The epitherial tissues enclosing the tip of the successor extend upward to about the level of the sebaceous gland, and along the epitherial tissue on the outside of the capsule in which the hair club is buried.

The level of the sebacenus glands is just at the base of the hair canal through which the tip of the young hair appeares on the surface of the body. The successors of large over hairs extend upward looking like to be carried on the back of the club hairs and to protect the predecessors.

9) So far as the animal is healthy the development of the successors are repeated periodically despite the outside temperature.

10) Both over and under hair are always enlarged in size to some degree by every replacement during the growth period of the animal. The under hair of the adult pelage showes no particular change in type in their generation. There is neither seasonal nor sexual change. However, it is apparent that dorsal under hairs are larger than abdominal under hairs in size.

11) There is no seasonal difference in large type over hairs. This comes from the fact that they are remained in the skin from the time of hair germs untill the shedding of club hairs covering about 4.5—5.0 months. Small type over hairs and under hairs the period of retention in the warm season is about 3.5—4.0 months being shorter than that of large over hairs, while the retention in the cool season lasts about 5.5—6.0 months being longer than that of the large over hairs.

12) After twelve months old, there is no increase of new hair follicle in number in the stratum germinativum.

On account of the retention of club hairs and the participation of successors, the hair follicles produce the generation of true hair bundles, namely coulpied hairs bundles and trio hairs bundles and these true hair bundles make pseudo hair bund-

les.

In the cold season, the largest number of hairs which are contained in the generation of true hair bundle developping from the same follicle is three. This is the trio hairs bundle.

There is no case in which all hairs of the trio hairs bundle are club hairs, i. e. T GnO, $G_{n+1}O$, $G_{n+2}O$. All hairs of the trio hair bundles are composed of two club hairs and a growing hair, i. e. $TG_nO$, $G_{n+1}O$, $G_{n+2}Y$ or $TG_nO$, $G_{n+1}O$, $G_{n+2}C$.

In the warm season there is no trio hairs bundle in the generation of hair bundles. They are coupled hairs bundles or solitary hair follicles. The coupled hairs are not of two club hairs, i. e. $PG_nO$, $G_{n+1}O$.

Accordingly the density of the hair in the cool season is about twice as much as that of the warm season.

13) The medulla of the under hair is of the chain shape and is composed of alternately arranged air chambers and medulla elements. The medulla in which the cells are arranged in a continuous line, has air chambers enclosed with medulla elements respectively. These air chambers are formerly occupied by the karyoplasm. Therefore every air chamber is formed in each cell.

14) In the over hair, the medulla fragments fix firmly on the innerwall of the cortex pipe at about regular intervals, and the microscopical observation of the longitudinal section reveals that they are confront, crossed each other or in net-like shape, forming zigzag air hollow in the medulla.

15) In the under hair stalk, there are many granular pigments in the medulla elements which are found between air chambers, while in the over hair stalk, the pigments are scattered uniformly in the cortex and the medulla.

16) The keratinization of the cytoplasm of hair cuticular cells may occur earlier than that of the cortex and the medulla. However, the keratinization of the cortex may occur simultaneously with the medulla.

17) The crimps of the under hair seems to be brought about by the cuticular cells which prevent cortex cells from the uniform arrangement in the peak of the hair bulb during the growth of the hair. Either in dorsal or in abdominal hair cover, the stalk of the under hair which has regular crimps is about thirteen micra in diameter, and the stalk of the under hair which is entirely surrounded with single cuticular coronal scales is about twenty micra in maximum. The blade of the under hair which has *"jomon" type coronal scales is about twenty-five micra in diameter in maximum.

* "jomon"= Cord marked

18) When the hair cuticular scales are consisted of single cuticular coronal scales, the medulla cells are arranged in a line.

19) The observation on the cross-sections of the hair stalk indicates the change of the singular cell to the pulural in the cuticle, the medulla-and the cortex-cell respectively. From this the boundary shifting from the under hair to over hair is considered very probable.

20) The hairs of the nutria are classified as follow

| | | whole length of hair | long diameter of hair stalk | long diameter of hair blade | percentage of number |
|---|---|---|---|---|---|
| | | cm | μ | μ | % |
| 1) first over hair | dorsum | 2.0-8.0 | 15-125 | 20-200 | 5-10 |
| 2) second over hair | abdomen | 1.5-5.0 | 15-85 | 20-120 | 1-5 |
| 3) cross-hair (cross-over hair, cross-under hair) | dorsum | 2.0-2.5 | 12-14 | 12-25 | 90-95 |
| 4) under hair | abdomen | 1.5-2.0 | 10-12 | 10-20 | 95-99 |

1) The first over hairs are the longest having no hair constriction. As a rule the hair blade is dark brown in color without agouti bands. There are some exceptions that have sub-apical light yellow bands.

2) The second over hairs are the next in length. The hair blade is wide with bright yellow agouti bands in the mid-blade. In the middle of the hair shaft there is a narrow constriction. In many of them the hair stalk is larger in diameter than that of the first over hair stalk.

3) cross-hairs,

It is possible to classify cross-hairs into two, i. e. over hairs and under hairs. The size of the stalk of the cross-over hair is the same as that of the cross-under hair. The hair blade of the cross-over hair is somewhat wider than that of the cross-under hair in diameter and the former has an agouti band while the latter is a blade with dark shade. The cross-under hair belongs to a large type under hair, and the features of hair blade, hair constriction and hair stalk are very much similar to those of the under hair. The width of the cross-hair stalk is intermediate between that of the under hair and that of the over hair and the name "cross-hair zone" should be applicable.

4) under hairs,

The under hairs being without the cross-hair zone are simply ordinary under hairs.

21) There is no development of new hair follicle after twelve months old in the nutria. At the several months after the birth, the density of the hair groups is decreased remarkably, but the density of the adult pelage is kept by about a half of that of juvenal pelage in degree on account of the development of postnatal sublateral hair follicles. However, at this time the density of the pelage is highest in the life of the nutria being provided with the fine under hair.

The density of the abdominal pelage is higher than the dorsal pelage and the quality of the hair in the former is finer than in the latter, so the skin for the fur cut along the mid-dorsal line should be prized. The abdominal skin of the juvenal animal is thin and brittle. From this reason the nutrias of above twelve months after birth and above four-five Kgs have utility value for the fur.

In many kinds of rodents there are normally two seasonal molts in a year, i. e. spring and autumn, and they supply us with fresh and fine winter pelages during

only about two months after December, while in the nutria there is continuous replacement of hairs throughout a year. However in the cool season the shedding of the club hair is delayed, increasing the density of the pelage above twice as much as the warm season.

Such prolonged retention occurs not only in winter, but also in late spring.

From the reason above mentioned we can understand that if this animal is kept in the pen supplied with fresh and cool water, and carefully treated, the superior pelts of higher value is maintained for considerably long period in a year.

22) The findings so far obtained in the present investigation are as follows:

1) The arrangement of hair follicles in the hair groups has been made clear.

2) The air chamber of the under hair is formed in the medulla cell in the place which is occupied by the nucleus.

3) The occurrence of the crimps in the wool of the sheep is said to be brought about by some chemical procedure but the crimps of the under hair in the nutria is resulted from the change of arrangement of cells.

4) Speaking morpho-genetically, the shifting of the under hair to the over hair is the shifting of singular cell to multiple cells.

5) There is no sexual age or seasonal difference in the under hair of the adult nutria.

6) There is no change in generation of hairs of the nutria according to the season, but during the cool season there occurrs very rare shedding of the hair. In the warm season 1—2 hairs are retained in a follicle while in the cool season 2—3 hairs are held.

7) In the most of the fur bearing animals there are regulary 2 shedding periods, spring and autumn in a year and so the pelting time is limited. However, the nutria replaces the fur all around the year. Accordingly we can control the pelting season by managing environmental condition artificially.

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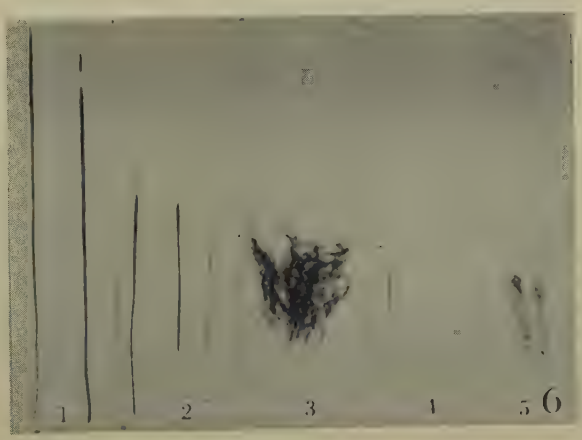
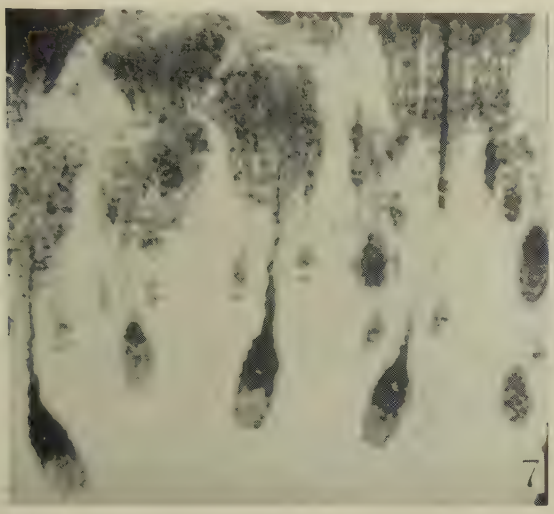
## 図　版　の　説　明

1. Nutria の1家族．牝牡成獣と仔獣（1腹）．
2. 水槽で游浴中の成獣．
3. 3カ月胎児．体重50瓦，実物⅖大，□印は資料採取部位．
4. 生後2日児，体重130瓦，実物⅖大，□印は資料採取部位．
5. 塩乾皮の毛面，面積24吋×17吋，成獣皮，凡て背割り調製．
6. Nutria の被毛，1は背部の第1上毛，2は第2上毛，3は分岐毛と下毛．4は腹部の第2上毛，5は分岐毛と下毛．




ÔTAISHI : Pelage of Nutria

7. 3カ月胎児のS切片より検鏡の毛叢．倍率は（顕微鏡倍率600）×（引伸器倍率5），鞘毛期の中央毛包を中心に毛栓期，球栓期の側毛包と毛芽の副側毛包を伴って叢林のようである．
8. 3カ月胎児のS切片，600×5，中央毛，側毛の3原毛は各々別個の毛管より出芽している，3原毛群．
9. 8と同様で，中央毛，側毛共に別個の毛管より発芽する．600×5.
10. 8, 9に同じ．
11. 生後2日幼獣背皮のR切片より，150×3．中央よりやや上位に最大毛包群の15生毛包群あり，その他7生毛包群型，3生毛包群型などあり．
12. 同上7毛包群型．150×5.
13. 同腹部毛群．150×3.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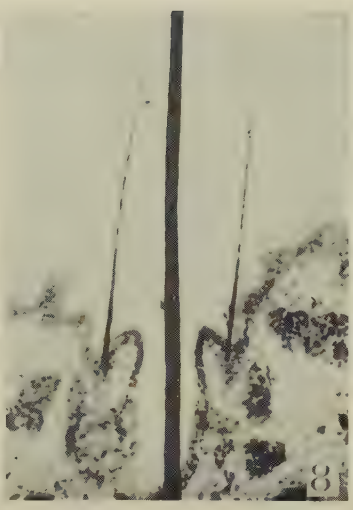
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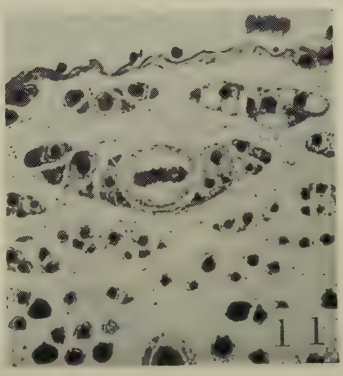
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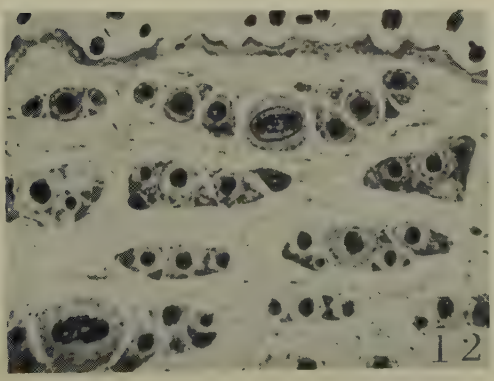
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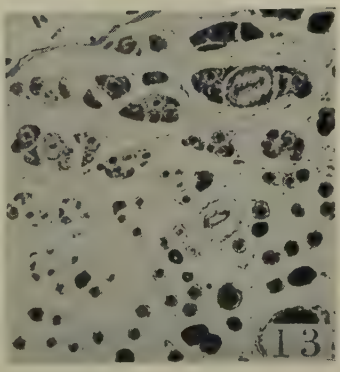
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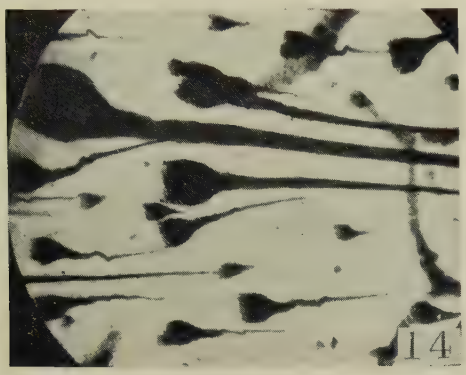
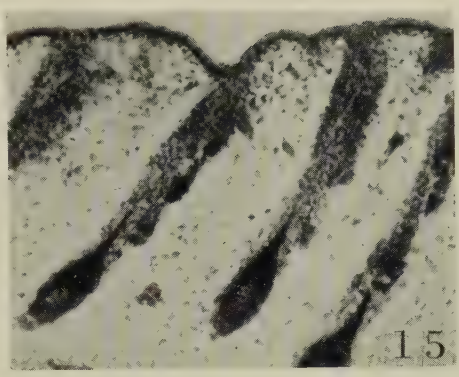
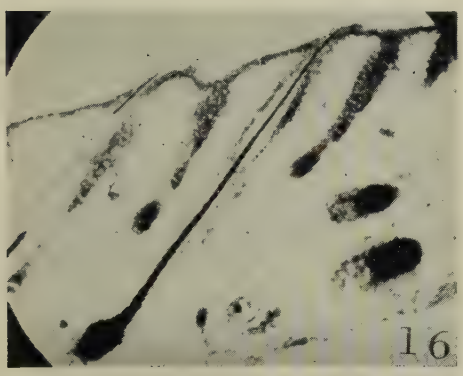
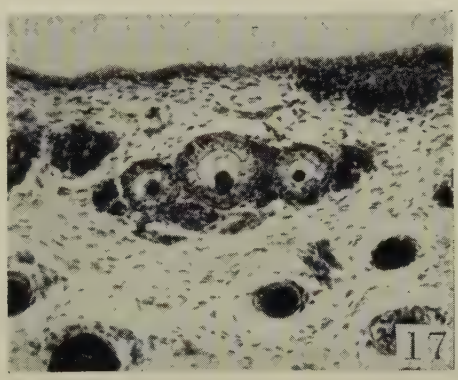
ÔTAISHI : Pelage of Nutria

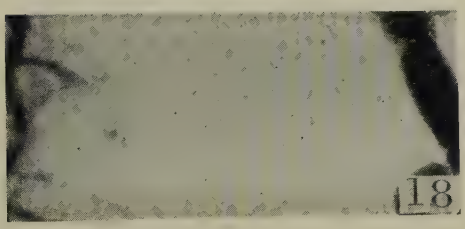
14. 3ケ月胎児，背部の染色無処理切片より．150×3.
15. 3ケ月胎児の背部L切片より，中央毛，側毛，副側毛の発生状態を側面より観察する．150×4.
16. 同じ背部L切片より鞘毛発芽の模様．150×3.
17. 3ケ月胎児の背部R切片，3原毛包は鞘毛，球栓毛の断面を表わすがその他は細胞の染着によって副側毛と推側して，15生毛包群と認める．150×4.
18. 3ケ月胎児皮の胸部，染色無処理で皮面より検鏡，血管の他皆無である．150×3.
19. 3ケ月胎児皮，染色無処理の背部L切片，鞘毛の毛管より発芽の模様あり．150×3.
20. 3ケ月胎児皮の胸部L切片，染色無処理，毛栓期の毛包を示す．150×3.
21. 3ケ月胎児皮の腹部L切片，染色処理，球栓期より鞘毛期に移る過程．150×3.

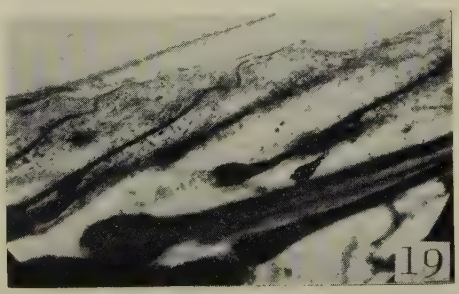


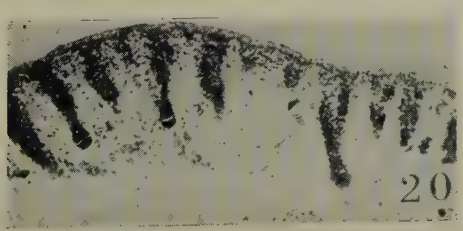



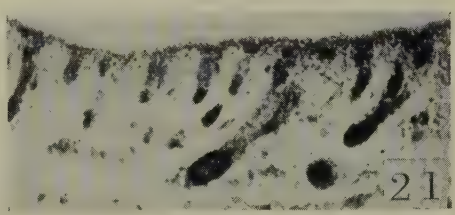



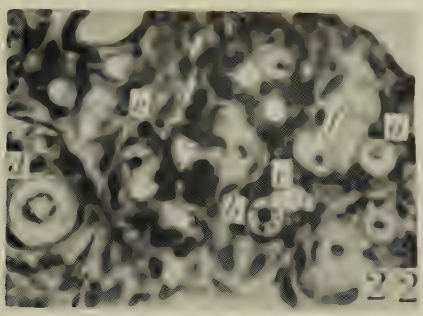



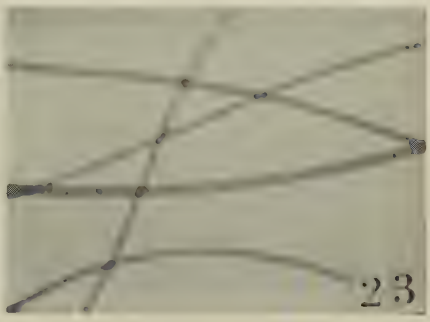



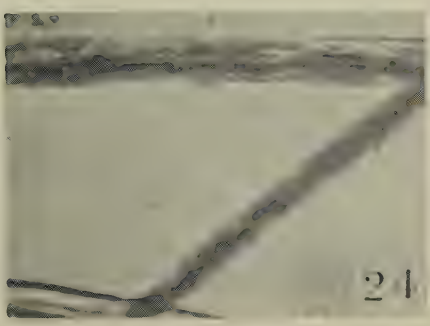



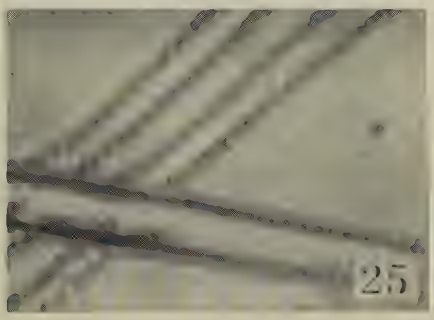

ÔTAISHI: Pelage of Nutria

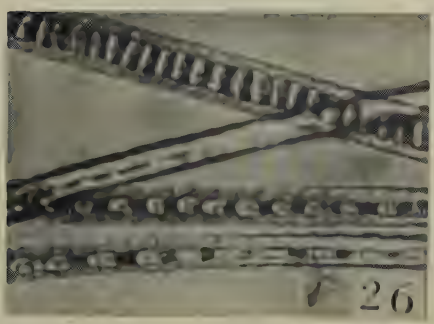
22. 成獣皮腹部毛群の脂腺位．600×3.5，aは下毛の毛柄，bは下毛の毛葉，cは下毛の毛根，eは下毛の毛芒，fは皮脂腺，dは小型上毛の毛柄を示す．
23. 腹部の下毛，分岐毛，150×3.
24. 腹部の下毛の毛葉，600×3.
25. 腹部下毛の毛葉の毛小皮鱗片スンプ標本，縄紋を表わす．500×3.
26. 腹部の下毛と分岐毛の毛柄の混在を表わす．600×3.
27. 腹部小型毛のスンプ標本．600×3.
28. Nutria の腹毛と merino 羊毛の比較，merino 羊毛は約 30 $\mu$, Nutria の柄幅は腹毛で 10—12 $\mu$. 600×3.
29. 背部下毛の芒先（スンプ標本），毛小皮は円筒状に連続している．600×3.

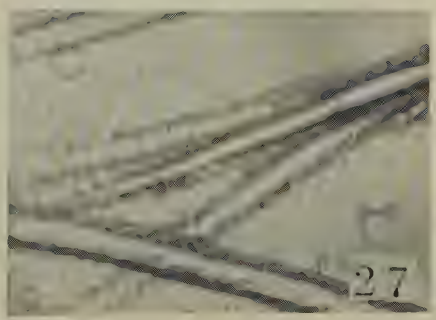


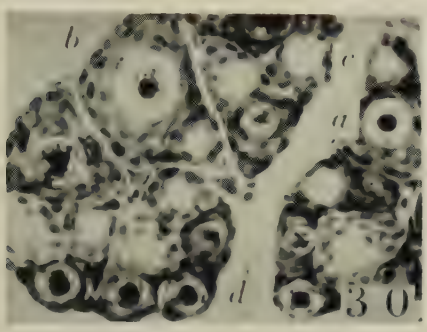



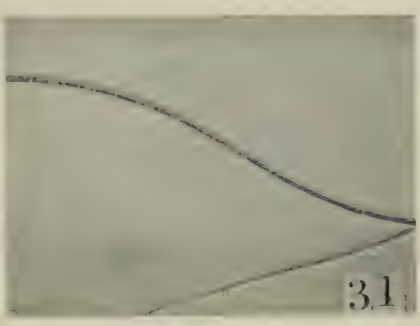



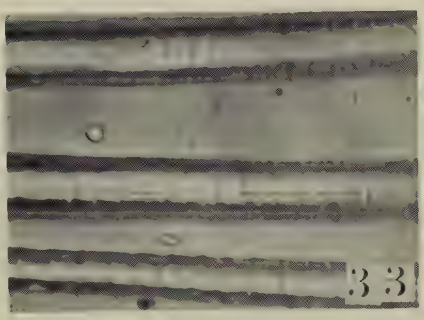



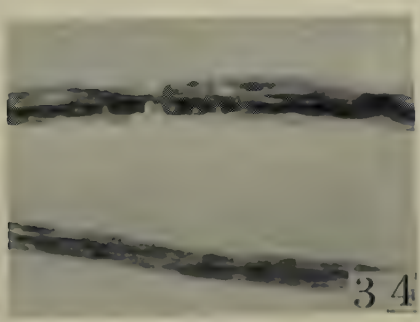
ÔTAISHI: Pelage of Nutria

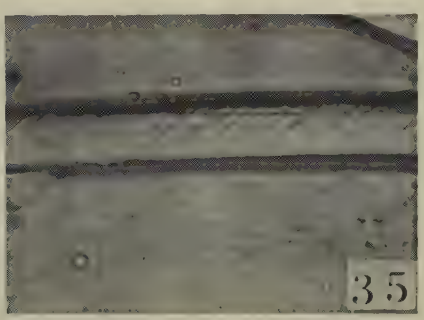
30. 背部下毛，600×3，a は毛柄，b は毛葉，c は毛根，d は毛葉の先端で毛芒，下端の黒色毛芒は2次副側毛包の新生したもので後継毛ではない．生後3ケ月幼獣．
31. 背部下毛繊維，毛柄部，150×3.
32. 背部下毛，600×3，髄質はやや彎曲内側に又は中心にある．
33. 背部下毛のスンプ標本．600×3，毛小皮は単冠状で円筒形の累積型となり最早明らかな縄紋型のものは少ない．
34. 背部下毛の毛葉，600×3，髄質は彎曲内側に偏つている．
35. 背部下毛の毛葉スンプ標本，彎曲内側に明らかに縄紋模様がある．
36. 生後2ケ月幼獣の背部毛群．600×3，これは脂腺下位で両側の毛束毛包は個々に集結している．表皮面では7毛束型以下の小型毛群であると推定され，両側毛は分岐域にある．
37. 背部下毛の毛根スンプ標本．600×3.

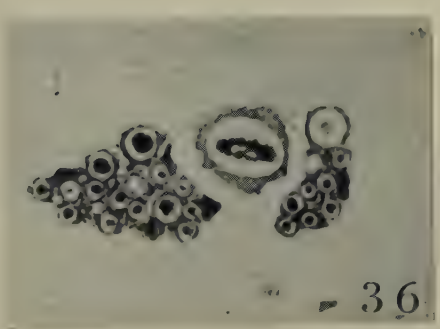


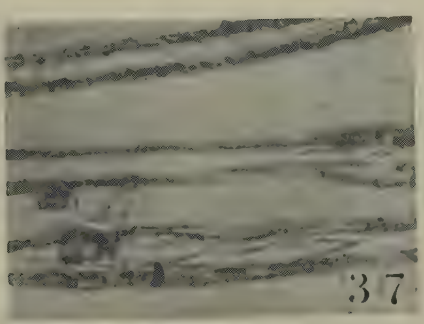





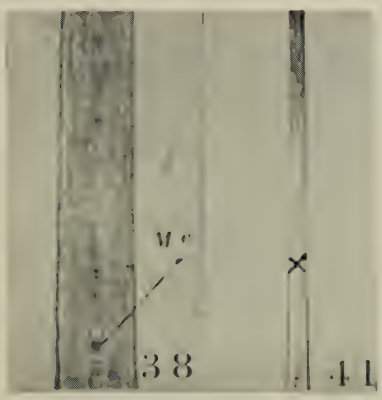



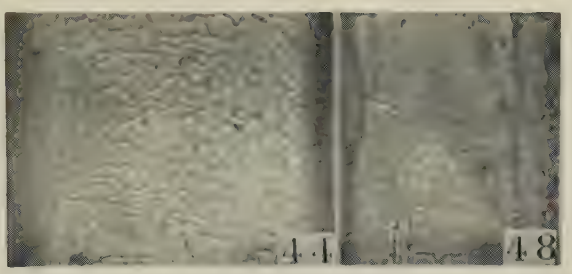



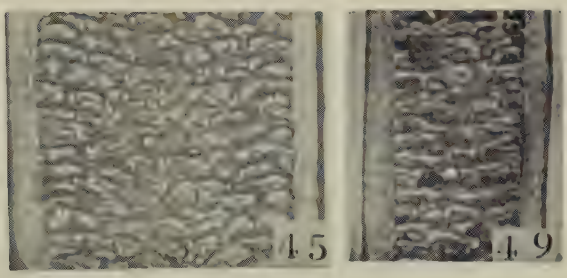



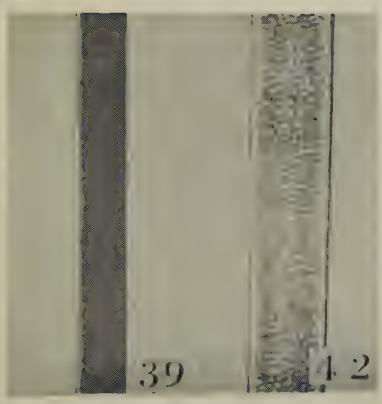

ÔTAISHI: Pelage of Nutria

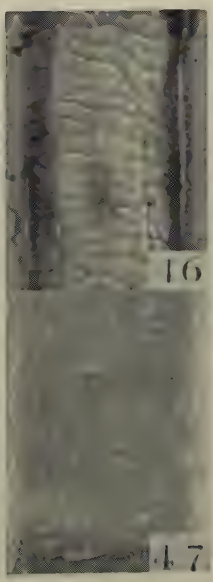
38. 背部第2上毛．毛長 2.6—3.0 cm 明度 3 の明葉毛の芒先で髄質が痕跡の断続を示している部分，褐色素，黄色素顆粒が混在する．Me 印は髄質の痕跡，なお髄質細胞は 1 列である．
39. 同毛柄．150×3.
40. 同毛根．150×3.
41. ×印は38の部分．毛芒．150×3.
42. 同毛葉の中央部．150×3，即ち明葉帯．
43. 同毛葉と毛頸の境．下方の暗黒部は褐灰素顆粒の混在多量を表わす．
44. 同毛葉のスンプ標本．600×3.
45. 同毛葉，葉幅 105 μ 600×3.
46. 同毛柄のスンプ標本．600×3.
47. 同毛柄，柄幅 72.9 μ 600×3.
48. 腹部第 2 上毛，毛長 2.1　2.5 cm，600×3，毛柄部のスンプ標本．
49. 同毛柄．600×3，柄幅 a＝90.9 μ，側幅 c＝63.9 μ
50. 同毛葉のスンプ標本，600×3.
51. 同毛葉，600×3，葉幅 a＝125.1 μ，c＝53.1 μ，腹部毛葉の髄質の幅は背部のそれに比して極端に狭い．

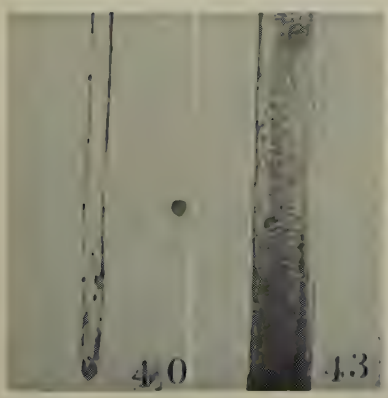


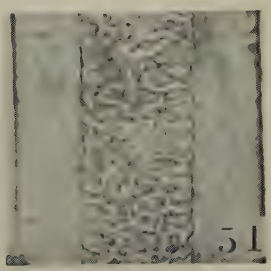



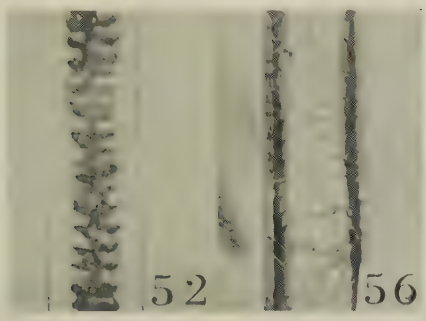



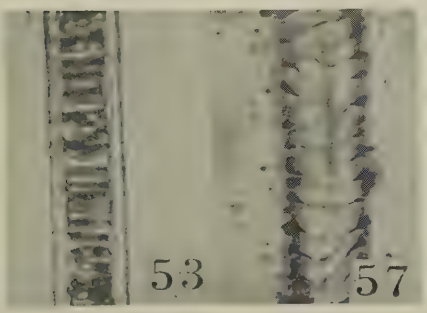


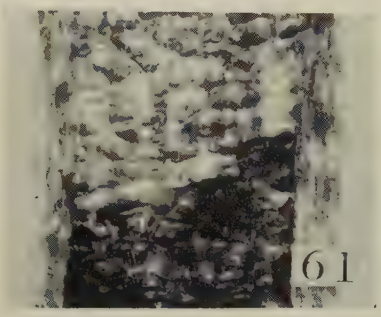



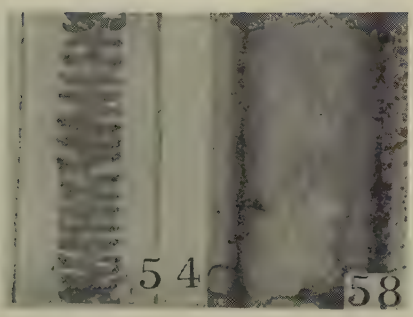

Ôtaishi Pelage of Nutria

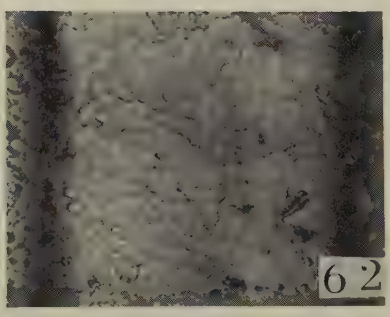
52. 小型第2上毛の毛葉，葉幅 36.9 μ，600×3.
53. 同毛柄，柄幅 31.5 μ，600×3. 52，53 は毛長 2.1～2.5 cm で分岐域を脱した小型明葉毛である．
54. 55. は毛長 2.6～3.0 cm の背毛の小型明葉毛の毛葉，毛柄である．葉幅 58.5 μ，柄幅 53.1 μ である．
56. 57. は 52（毛葉），53（毛柄）のスンプ標本で毛小皮面を表わす．600×3.
58. 59. は 54（毛葉），55（毛柄）のスンプ標本，600×3.
60. 背部第1上毛，暗葉毛，毛長 7.5～8.0 cm 毛葉のスンプ標本，600×3.
61. 同毛葉，暗葉，葉幅 a＝161.1 μ，明帯なし，600×3.
62. 同毛柄のスンプ標本，600×3.
63. 同毛柄，柄幅 a＝134.1 μ.

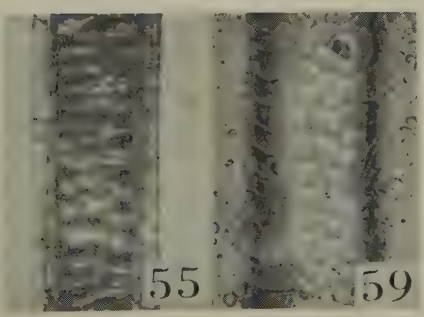


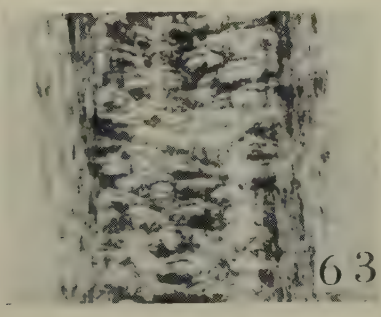


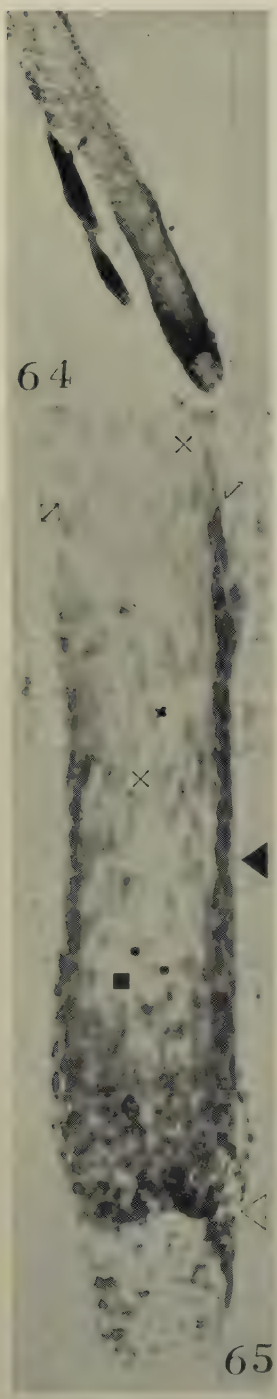



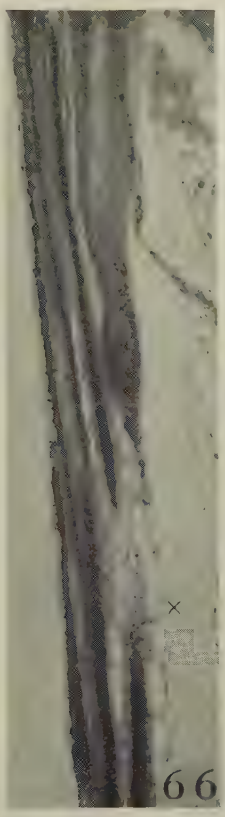



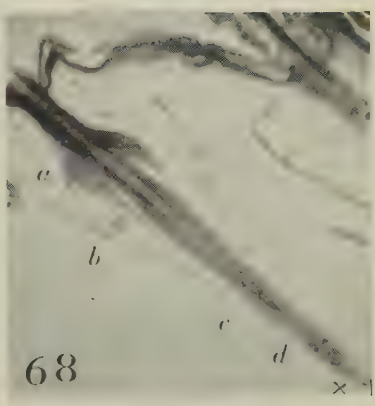



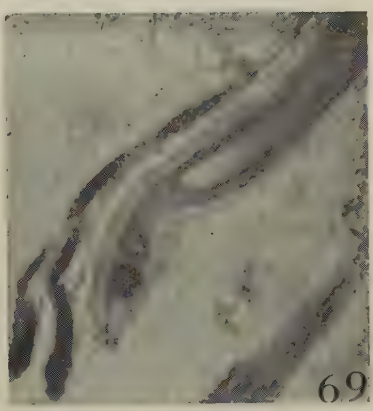

ÔTAISHI : Pelage of Nutria

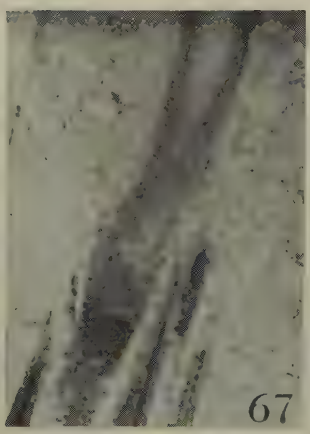
64. 背部上毛の毛包の萎縮開始，成毛球即ち棍状毛球化の開始，150×3.5.
65. 同毛包の郭大観察，600×3.5，衰退期初期の上皮索と毛乳頭を表わす．×印は角化細胞の核で紡錘形，■印は角化停止後増殖の核で球形，▶印はこの辺りが上皮索の角化停止域を，▽印は内根鞘を示す．▷印は残留色素を示す．濃染部は角化せず，むしろ分裂増殖した新鮮な細胞である．これが次代の毛芽となる毛母基である．
66. 背部上毛の毛包の萎縮中，×印は Haarstengel を示す，150×3.5.
67. 背部上毛の再生期の初めで郭大すれば72のようになり，中央部上位の円形細胞を含む毛母基は活発となる．
68. 小型第2上毛の衰退期，上皮索 d は萎縮中である．a は脂腺，b は起毛筋，×印は Haarstengel である，600×3.5.
69. 背部上毛の休止期の毛包，71にその休止乳頭とその上位に休止毛母基が現われている，600×3.5.
70. 背部上毛の再生中の毛包，150×3.5，74にその乳頭と毛母基を示す．乳頭は紡錘形になり Haarstengel に沿って下降中である．



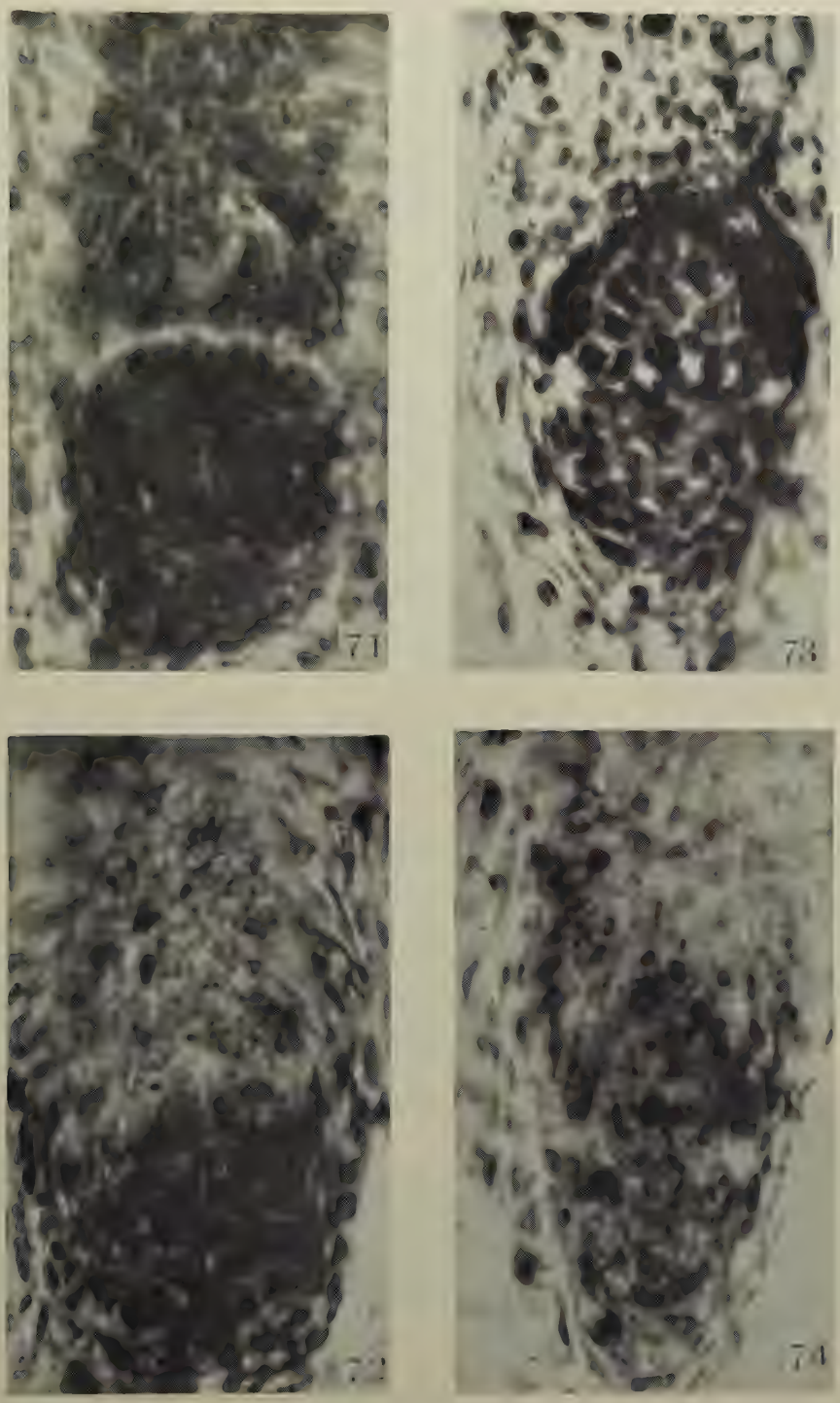



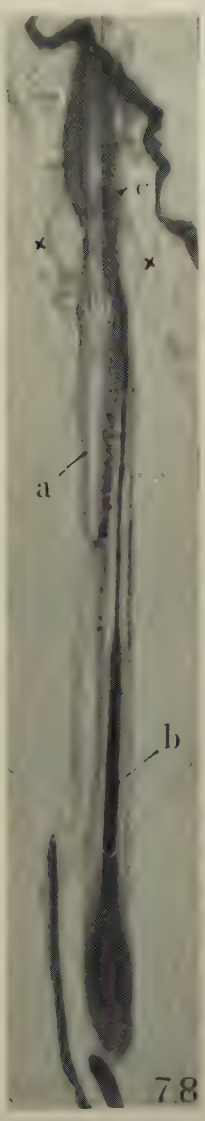
ÔTAISHI : Pelage of Nutria

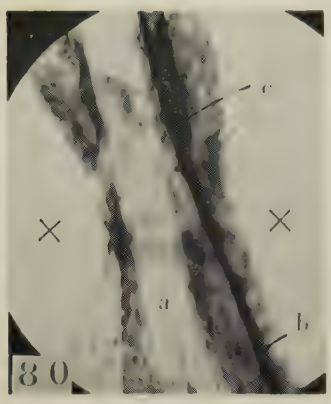
71～74，これ等は上述のように乳頭，600×8，毛母基の休止期より再生期を示す．

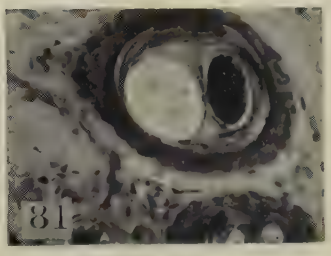


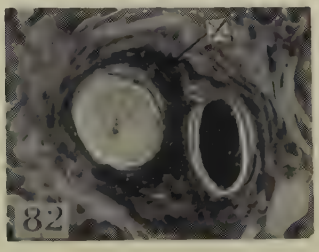
Ôtaishi Pelage of Nutria

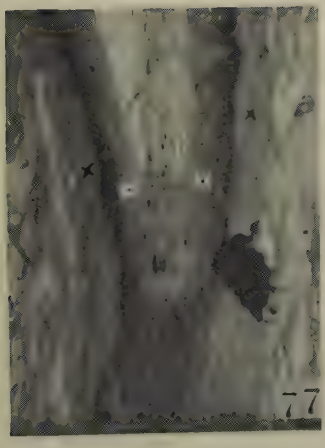
75. 背部上毛の換毛，150×3，後継毛は球栓毛より鞘毛に発育中．
76. 更に鞘毛期より表皮面に現われて幼毛の列に入らんとする，50×4.
77. 棍状毛根，600×5，半角化状態の被膜細胞（⊙印，capsule cells）と刷子状に角化した粗面の毛棍球とが緊密に嚙み合っている．
78. 76 の郭大図，150×3，a は棍状毛根，b は後継幼毛，c は毛管の坑道，×印は脂腺を示す．
79. 第 1 上毛（暗葉毛）の毛球，600×3，毛葉が発生中の毛球，×印は毛小皮の核が明瞭である．
80. 78 の換毛中の脂腺位の像，×印は脂腺，a は棍状毛根，b は幼毛の毛芒先，c は毛管の坑道，後継毛は成毛根に平行して発育して，成毛に触れていない．毛管内に出て初めて成毛と会している．これに似類の状態の毛包横断面である次の 81，82，83 も明らかである．
81. 表皮面の対生毛の状態を表わす，600×3.
82. これでは毛管の下底においてヘマトキシリン色素が多く沈澱している（×印）．成毛と幼毛は明らかに離れている，600×3.
83. 対生毛束の皮脂腺下位に当る成毛根を支えている被膜と幼毛とを囲遶している細胞は活発である，600×3.

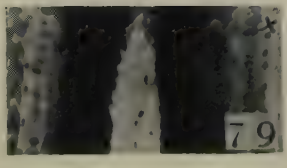


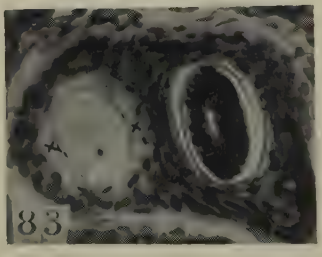






ÔTAISHI: Pelage of Nutria

84. 腹部下毛のスンプ標本，600×5.

85. 腹部下毛，柄幅 11.7 μ，600×5.

86. 下毛の毛球断面，600×7.

87. 腹部下毛の毛球の上位，600×7，毛柄，×印は毛小皮核，未角化柄幅 17.1 μ，角化後柄幅（a＝b）13.5μ，毛小皮核は 1 個である．

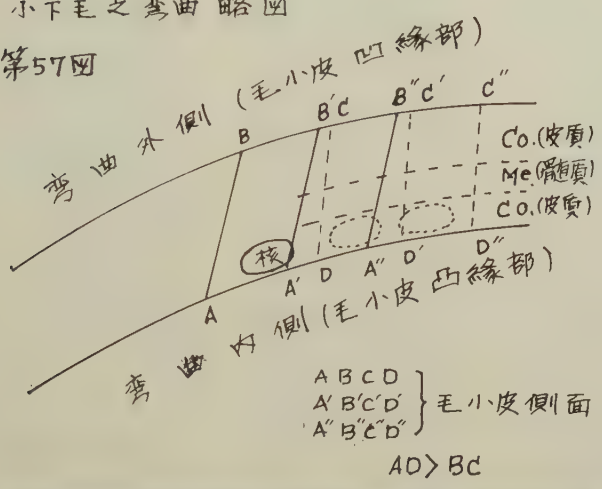
88. 腹部下毛の毛包，毛球上位，a は髄質細胞の核，b は皮質細胞の核，c は毛小皮細胞の核を示す，600×4.

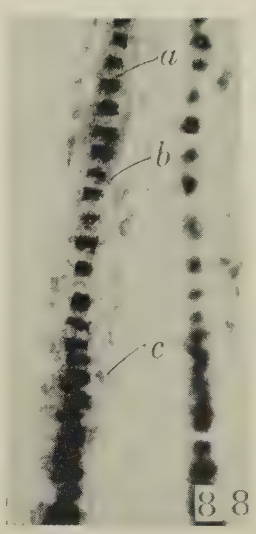
89. ×印は腹部下毛の毛球上位で毛小皮核 1 個，(a 印)，毛柄の未角化部，幅 13.5 μ，角化後柄幅 9.9 μ で最小位の下毛である，600×7.

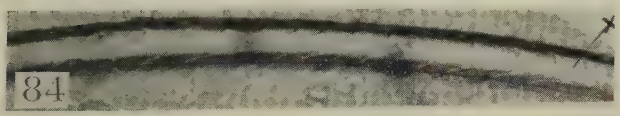
90. 腹部毛群，脂腺下位，600×7，×印は下毛の毛柄（角化後）の ℝ 断面である．86，87 と比較して毛球より上方に至る細胞の変遷を対照する．

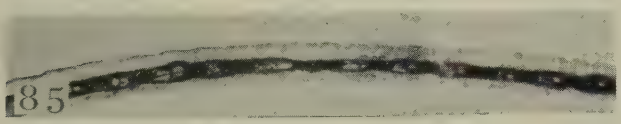
小下毛之彎曲略図

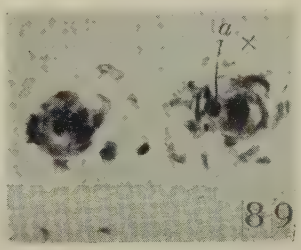
第57図



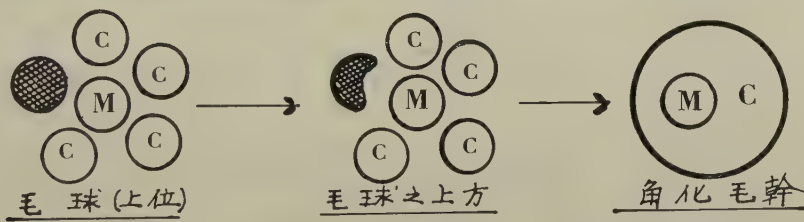



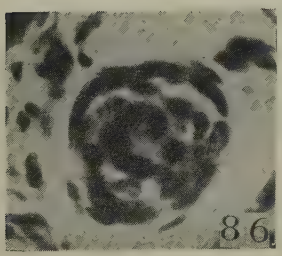



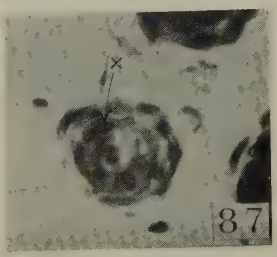



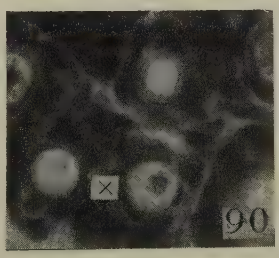




第58図.

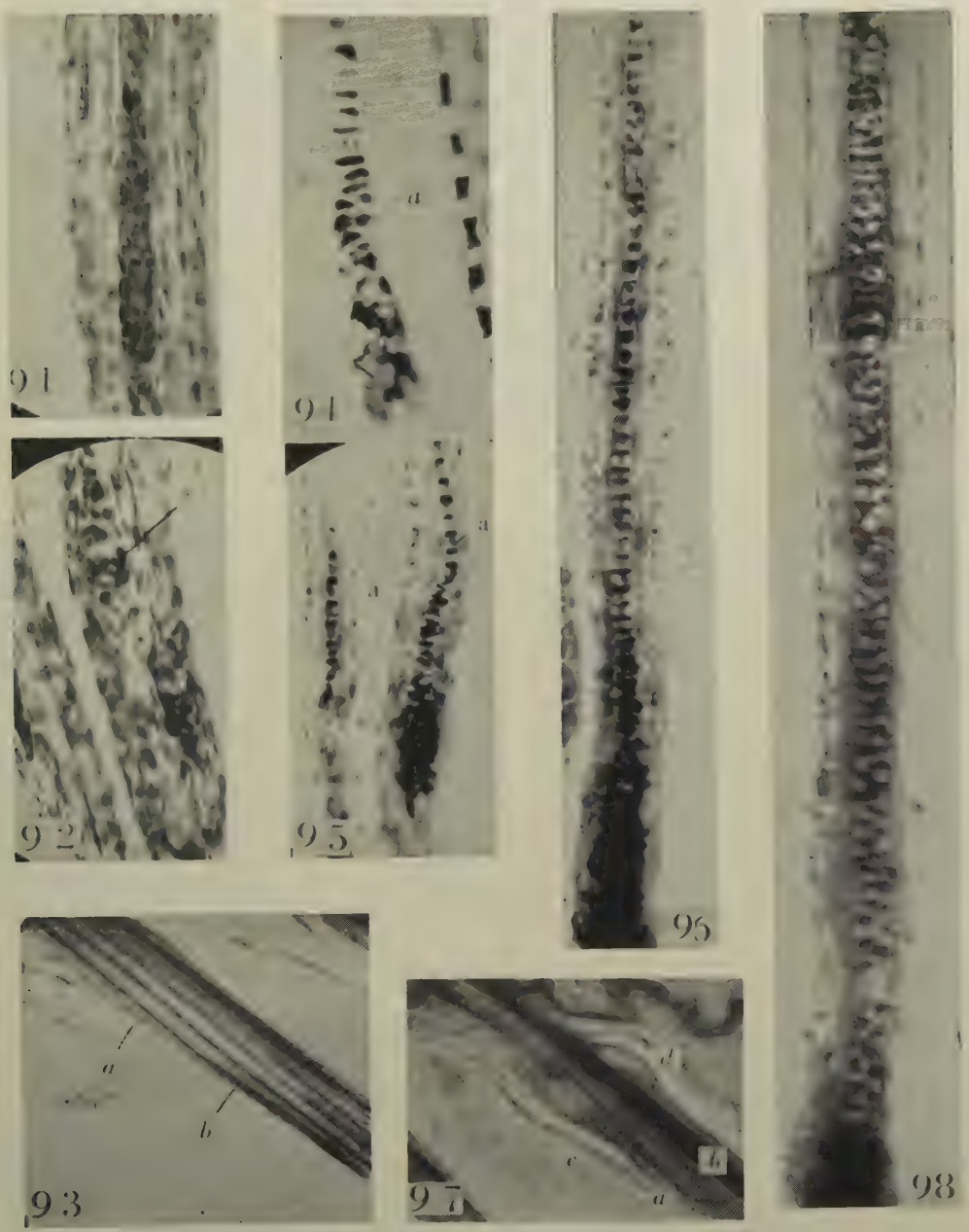


91. 下毛毛芽の毛球の下降を表わす，600×3.
92. 下毛毛包の衰退期を表わす，600×3，×印は乳頭と毛母基を示す.
93. 下毛の幼毛初期，600×3，a は成毛根，b は後継幼毛を示す.
94. 背部下毛の染色無処理，600×4，黒色は多く髄質細胞中の色素を表わす．毛球上位では細胞も数個輻輳しているが徐々に1列になつて上昇している．染色無処理なので核は不明である，
95. 背部下毛の染色処理せしもので，94 と異り細胞核は着色している．a 印は細胞核を示す，600×3.
96. 背部上毛の毛柄部成長中の毛包，L 切片，600×4，角化部位は a=b c=d，又は a>b c>d，b=27.9 μ，d=11.7 μ.
97. 生後2ケ月幼獣の背皮の L 切片，150×3，a は成毛根，b は後継幼毛，c は起毛筋，d は脂腺，$G_1O$. に対して後継毛 $G_2C$ は肥大している．所謂 step-up を表わす．分岐毛以上と推定する.
98. 背部上毛，毛柄部成長中の毛包，L 切片で側幅面を観る．角化部位 b=33.3 μ，d=15.3 μ，600×4.




ÔTAISHI : Pelage of Nutria

99. 背部上毛，明葉毛の毛葉部成長中の毛包，600×4，葉幅面を S 切片で観る．a＝85.5 μ，b＝53.1 μ.

100. 背毛上毛の毛柄部成長中の毛包，毛柄の正幅面を S 切片で観る，600×4.

101. 背部第 2 上毛，毛柄の角化部位の R 切片を観る，600×4，角化，脂腺位 a＝92.7 μ，b＝49.5 μ c＝63.9 μ，d＝17.1 μ.

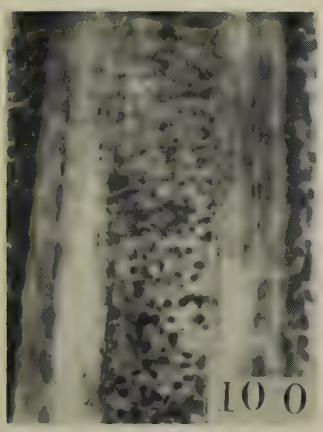
102. 背部明葉上毛，毛葉部成長中の毛包，葉側幅面を L 切片で観る．b＝80.1 μ，d＝20.7 μ，600×3.

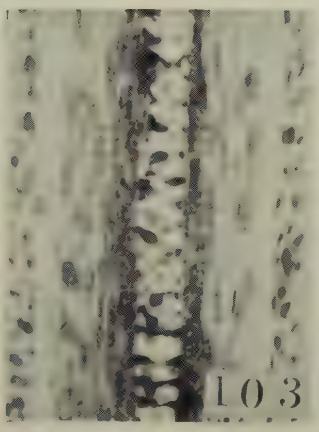
103. 背部上毛，毛柄部成長中の毛包を L 切片で観る．柄側幅 62.1 μ，推定柄幅98.4 μ，600×3.

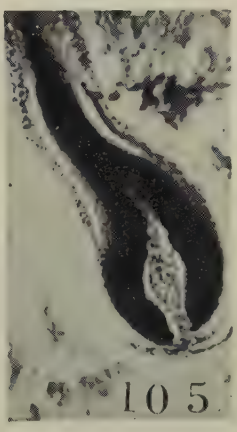
104. 上毛の毛包，葉明帯成長中の毛包である．切断不正のため，即ち斜断の結果先き細りとなり栓毛期の毛球のようであるが，細胞の変化の凡そは判明する，150×3.

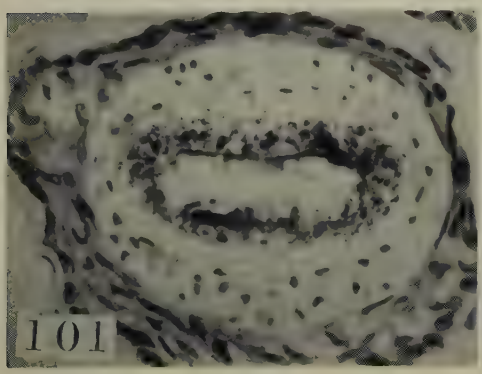
105. 毛葉部成長中の毛包で，104 と同条件にある．150×3.

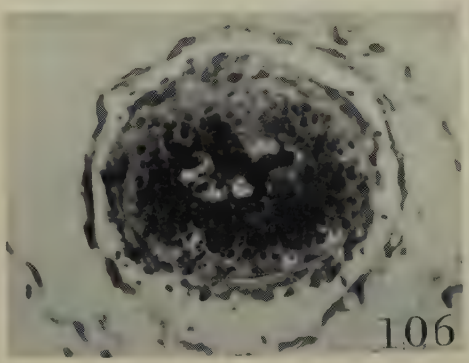
106. 101 の背部第 2 上毛の毛柄の未角化部位で，毛球上部で a＝80.1 μ，b＝62.1 μ，c＝44.1 μ，d＝20.7 μ，標本作製中萎縮の痕跡あり，600×4.

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107. 第1上毛の暗葉毛 ℝ 切片，600×4，脂腺上位の角化部位，a＝139.5 μ，b＝58.5 μ，c＝89.1 μ，d＝11.7 μ.

108. 同上の毛球上位である，a＝143.1 μ，b＝94.5 μ c＝71.1 μ，d＝17.1 μ，600×4.

109. 背部第1上毛，明葉毛の葉明帯の ℝ 切片，脂腺上位の角化部位，a＝215.1 μ，b＝74.7 μ，c＝164.7 μ，d＝17.1 μ，600×3.

110. 背部小型毛，毛柄成長中の毛包，600×4 角化，脂腺位，a＝36.9 μ，b＝27.9 μ，c＝18.9 μ，d＝8.1 μ.

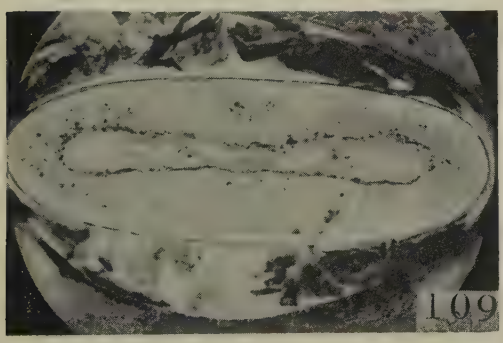
111. 同上未角化，毛球上位，a＝40.5 μ，b＝31.5 μ c＝11.7 μ，d＝11.7 μ，髄質の核は2個明瞭である.

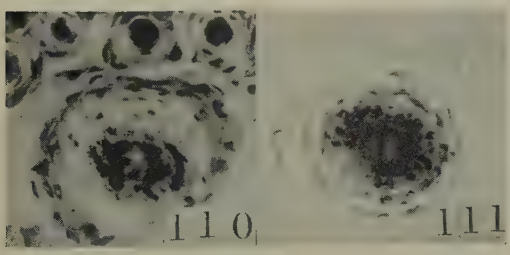
112. 腹部上毛の葉明帯の ℝ 断面，601×3，角化部位の断面に乳濁色に近い淡黄色顆粒を表わす.

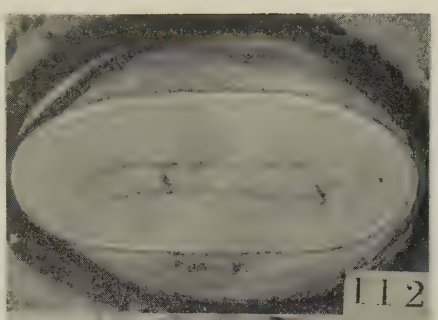
113. 毛葉の葉明帯，未角化，毛球上位，600×3，112の未角化部位ではない. a＝98.1 μ，b＝65.7 μ 毛小皮，皮質，髄質等の細胞核形質は濃染している.

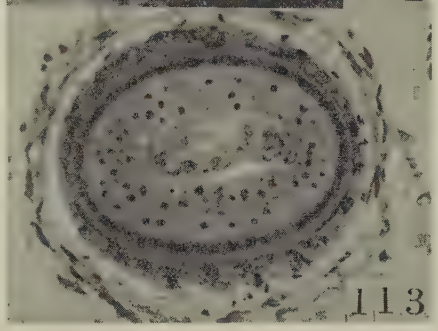
114. 腹部小型上毛の毛柄毛包の ℝ 断面，角化，脂腺位，a＝b＝27.9 μ，c＝d＝15.3 μ，600×5.5.

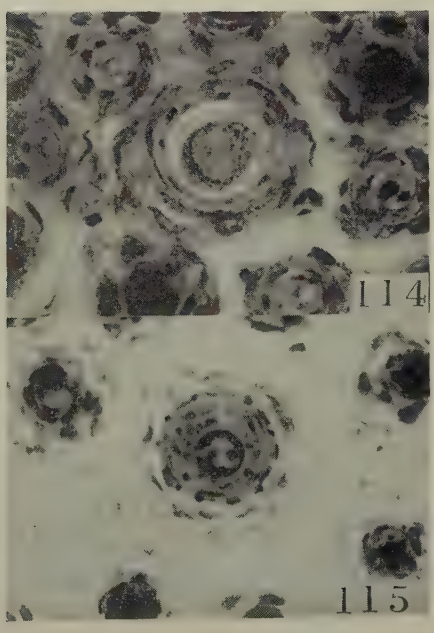
115. 同上腹部毛包の毛球上位 ℝ 断面，600×5.5，a＝b＝29.7 μ，c＝d＝11.7 μ，髄質の核は1個明白で左方の1個は焦点を変えると現われ，交互に出没する．髄質の細胞は正しく1列に並んでいない．おそらく側面からはジクザグに列が乱れている.

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116. 成獣背部毛群の S 切片，600×4.5，これは側毛束の1部であるが，凡て毛群は扇形に降下して皮脂腺位より上方で毛束を結成して分離し，降下すると共に毛包は合流して接着して生毛包の発生時と同様に毛球部は接触して来て，毛群の根幹である3原毛包が相接して最深部にとどまることはℝ切片と対照して明らかである．

117. 12月の成獣背毛群，600×4，最大15毛束群型に属する毛群であるが，前方の小毛束2個を加えて16毛束群である．中央毛，側毛共に幼毛で，同じ毛管に対生していた成毛が脱落した直後のように毛管は緩んでいる．右側毛はやや毛管が引き緊っているが，いづれも同様に上毛の毛葉下位の横断面を表わしている．切断深度は表面よりやや下位にある．

118. 7月の成獣背毛群，600×4，最大15毛束群型に近い13毛束毛群である．中央毛，側毛共に成毛で毛根の部分の断面を示している．切断深度は表皮面に接している．

119. 生後8ケ月腹毛群のℝ切片，600×4，中央毛，左側毛は共に上毛の葉明帯の断面を表わして幼毛であり，共に成毛が脱落後間のないことを物語っている．右側毛は成毛で断面は毛根部を表わしている．前方の5毛束は同じ毛群系のものではない．

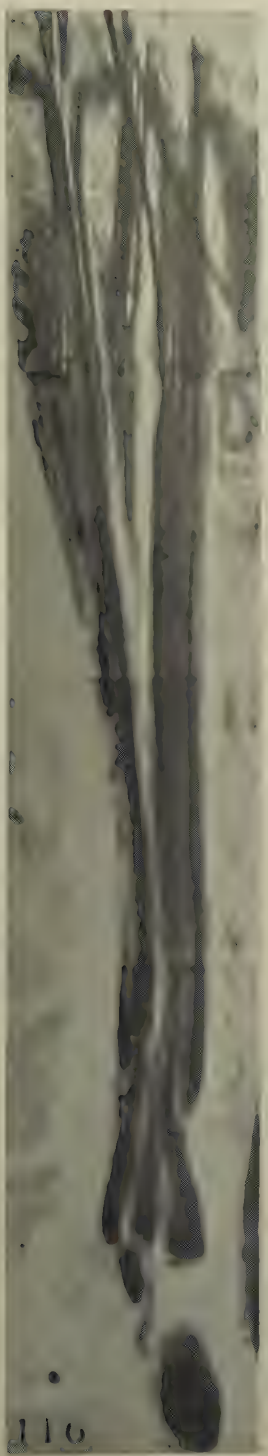
120. 4ケ月幼獣の腹部毛群，600×3.

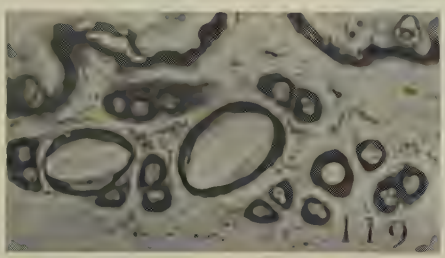
121. 12月成獣背部毛群の1部，600×6. 下毛包の3連生毛包を表わす，即ち同じ毛包に $G_nO$, $G_{n+1}O$, $G_{n+2}C$. の3毛世代を含んでいる．よつて3連生毛包の記号を $\underline{TGnO, G_{n+1}O, G_{n+}\ C}$. とする．

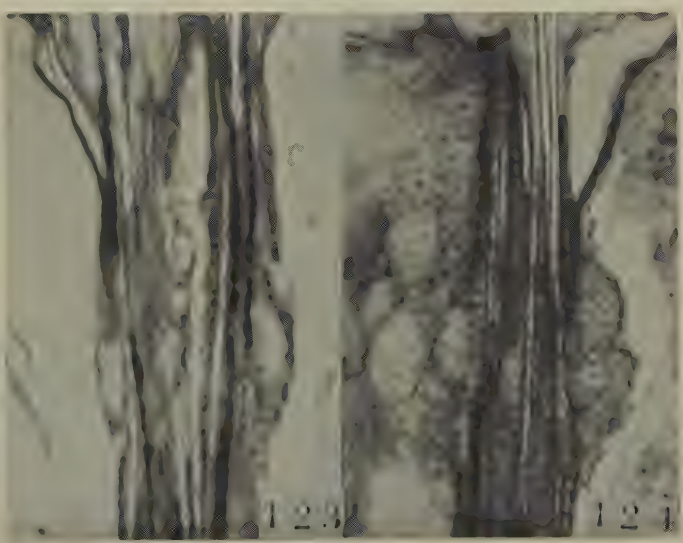
122. 欠除．

123. 背部毛群の S 切片，600×4.5，脂腺位の上方では2毛束に分離している．

124. 脂腺は毛束の外にはみ出している．600×4.5.



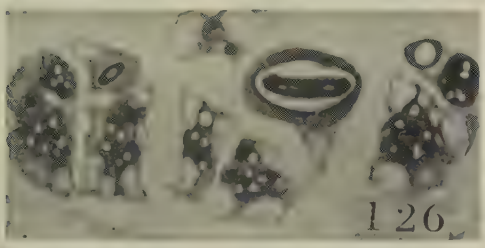


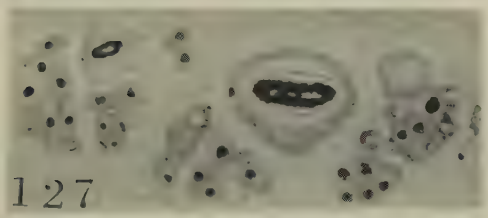


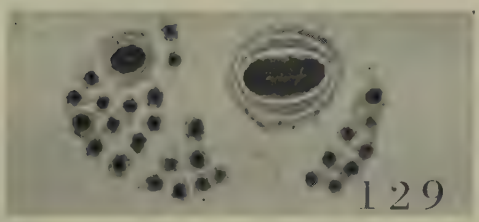

ÔTAISHI | Pelage of Nutria

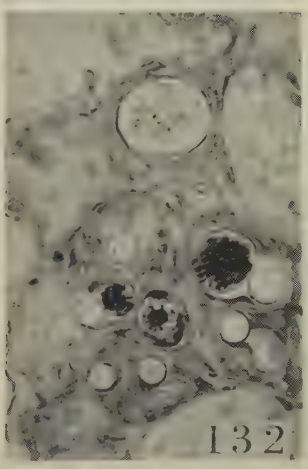
125～132. 本論中に詳述している．右側の毛束は成毛 6，発育毛 3，合計 9 毛結束の毛束であるが，脂腺位では 132 のように対毛 2，発育毛 1 は青毛で毛柄部を表わしている．毛管は弛み成毛が脱落後間のないことを表わしている．

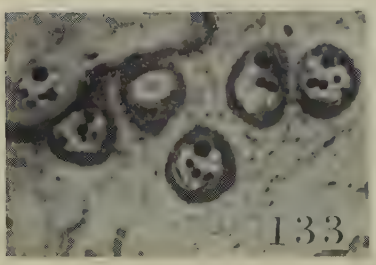
133～134. 12月成獣の背部毛群，150×3.5，600×3，中央毛のみが上毛で他の毛束の生毛後継毛は凡て分岐毛と認められる．冬期の毛束は毛量多く 134 においては 13 と 14 毛結束の 2 毛束である．その多くは成毛で占められている．







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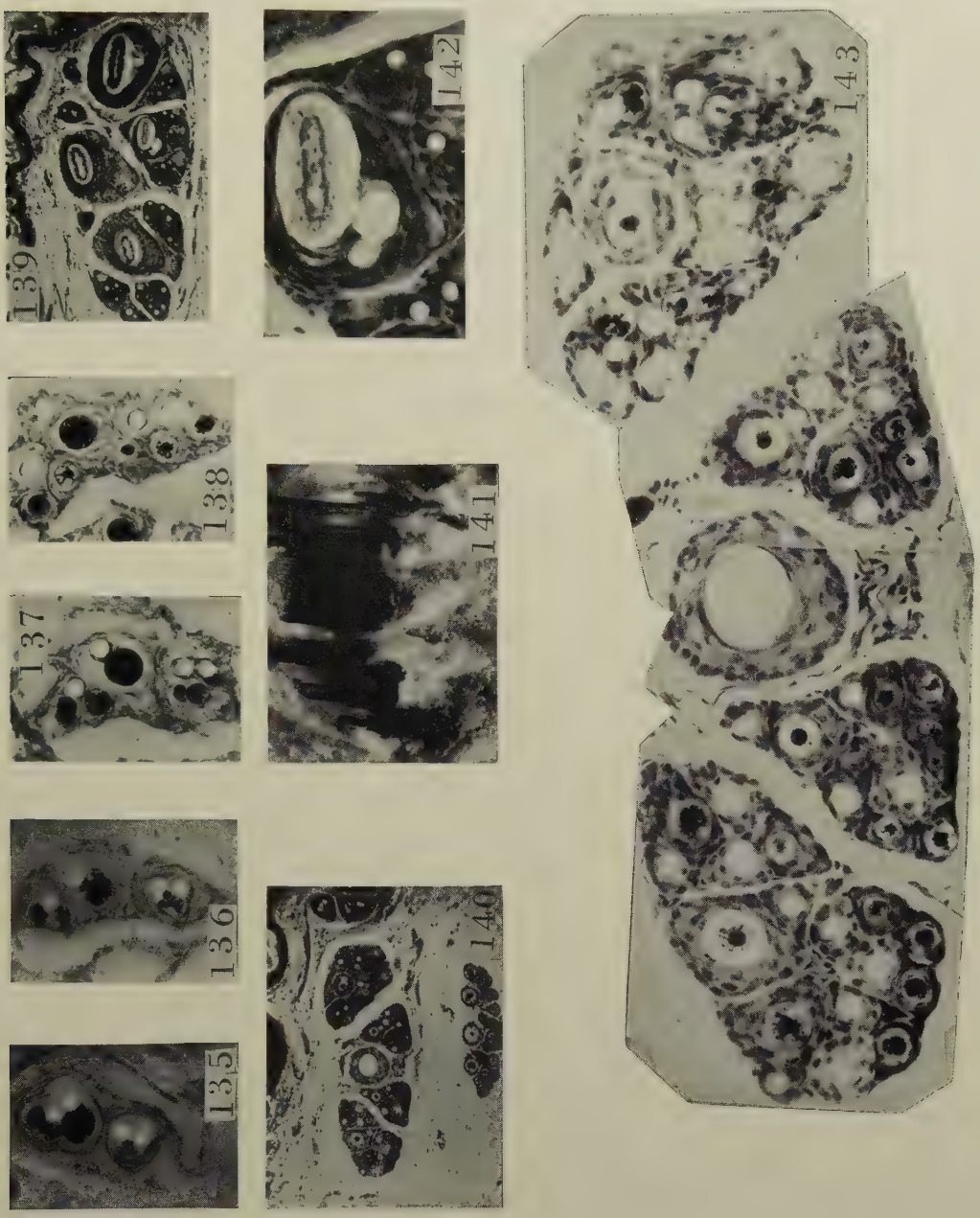
135～138, 生後2ケ月幼獣の毛群の解体を示す．600×3，皮面に接する毛束では発育毛1，成毛2，計3毛結束のようであるが，脂腺位，乂その下位と順次に切り下げるに従って最後に成毛2，青毛1，幼毛2の5毛が現われ，2次副側毛包の新生を物語っている．

139, 142. 3カ月幼獣の背部毛群，150×3，600×3.2 これは脂腺位で明らかに毛束を数え得ないが，副側毛と見做される毛包に $\underline{TG_1O}$, $\underline{G_2O}$, $\underline{G_3C}$ の3連生毛束があり，この$G_3C$. は明葉毛の葉明帯の断面で第2上毛なることが判明する．

140, 143. 生後3ケ月の背部毛群，150×3，600×3.2，これは脂腺下位を表わして，毛束数は不明であるが中央毛のみ上毛で，両側毛は分岐明葉毛程度の小型毛である．下方の黒色下毛は2次副側毛の新生毛包である．

141. 成獣の背部毛群，鞣毛皮より検鏡，150×3.

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# Die Anwendungsmöglichkeiten der Formoltitration in der Molkereipraxis

Yûzô Anagama und Takayasu Kami

*Institut für Tierzuchtlehe der Fakultät für Fischerei- u. Tierzuchtlehre an der Universität Hiroshima, Fukuyama, Japan*

(Tabelle 1)

## (I) EINLEITUNG

Das Milchvieh wird seit Jahren auf höhere Milchmenge und Fettleistung gezüchtet, wozu die leicht durchführbare Fettbestimmung nach Babcock oder Gerber die Grundlage bildet. Aber in den letzten Jahren ist die Bedeutung des Eiweissgehaltes der Kuhmilch mehr und mehr in den Vordergrund gerückt worden, und viel Mühe wurde aufgewendet auf die Entwicklung einer möglichst einfachen und billigen Routine-Methode zur Bestimmung des Milcheiweissgehaltes.

Die einfachste und billigste Methode ist wohl die seit Jahrzehnten bekannte Formoltitration. Richardson *et al.*[1] sagten, dass die Werte der Formoltitration geeignet waren, auf der Basis entweder von Fett und Protein oder von Fett und fettfreier Trockenmasse die Milch zu bezahlen. Schulz *et al.*[2)3)] berichteten, dass die Formoltitration als Eiweiss-Schnellbestimmung in der Molkereipraxis für verschiedene Zwecke angewandt werden konnte. Schober & Fricker[4] untersuchten insbesondere die Abweichung der Werte der Formoltitration von denen, die nach Kjeldahl gefunden wurden, und fanden, dass bei Kolostralmilch die Abweichung hoch war, dass altmelke Milch extrem niedrige Eiweisswerte zeigte und dass zwischen dem 30. und 300. Tag der Laktation Formoltitration und Kjeldahlbestimmung gut übereinstimmten. Kay[5] sowie Tentoni *et al.*[6] kamen zu dem Schluss, dass die Formoltitration unter den verschiedenen neuesten Routine-Methoden für Milcheiweissbestimmung die geeigneteste sein möchte.

Kofranyi[7] veröffentlichte eine neue Schnellmethode zur Ermittelung des Eiweissgehaltes der Kuhmilch, und Have & Mulder[8] berichteten, dass im Augenblick die Kofranyi-Methode eine der best geeigneten für Reihenanalysen in einem einfach eingerichteten Laboratorium sein dürfte.

Schober & Hetzel[9] berichteten eine einfache, kolorimetrische Methode zur Milcheiweissbestimmung mit dem Farbstoff Amidoschwarz 10B, und Steinsholt[10] sowie Raadsveld[11)12)13)] modifizierten und vereinfachten diese Amidoschwarz-Methode. Beinahe gleichzeitig führte Udy[14] eine einfache, kolorimetrische Milcheiweissbestimmung mit dem Farbstoff Orange G durch, und diese Methode wurde messtechnisch verbessert und vereinfacht von Ashworth & Seals[15], Ash-

WORTH *et al.*[16] sowie TREECE *et al*[17].

In den Niederlanden, um den Eiweissgehalt der produzierten Milch anzugeben und die Eiweissbezahlung der Anlieferungsmilch einzuführen, wird ab 12. Mai 1957 die gesamte in der Provinz Friesland gewonnene Milch in einem Zentrallaboratorium in Leeuwarden auf Eiweissgehalt untersucht. Als Methode dient hier die modifizierte KOFRANYI-Methode. Die zweite Provinz, die die gesamte Anlieferungsmilch zum Zwecke einer Eiweissbezahlung auf Eiweissgehalt untersucht, ist Gelderland-Overijssel, die am 1. Oktober 1958 ein Zentrallaboratorium in Zutphen einrichtete, das jedoch mit der Amidoschwarz-Methode arbeitet[18)19)].

Unter den heutigen Verhältnissen in Japan ist vielleicht die Formoltitration die geeigneteste als Routine-Methode. Zur Prüfung der Anwendbarkeit der Formoltitration in der Molkereipraxis verglichen wir die Werte der Formoltitration mit denen, die nach der KJELDAHL-Methode gefunden wurden. Es existieren verschiedene Formeln zur Errechnung des Milcheiweisses in Prozenten aus dem Fettgehalt der Kuhmilch. Wir verglichen auch die Werte nach der von GAINES[20] aufgestellten und nach der von OVERMAN *et al.*[21] aufgestellten Formel mit denen, die nach der KJELDAHL-Methode gefunden wurden.

## (II) MATERIAL UND METHODE

Die Untersuchung wurde ausgeführt an gemischten Milchproben, welche aus 3 Molkereien der Stadt Fukuyama in Hiroshima-ken stammten. Die Milchproben wurden in jeder Molkerei aus einem Pasteurisationsapparat von etwa 360 Liter Inhalt vor Erhitzung entnommen.

Die Untersuchung wurde in der Zeit von Anfang Januar bis Anfang März 1960 durchgeführt.

Die Fettbestimmung der Kuhmilch wurde nach GERBER ausgeführt. Die Milcheiweissbestimmung erfolgte nach dem Mikro-KJELDAHL Verfahren. Die Formoltitration wurde nach PYNE[22] ausgeführt. Die Formel von GAINES und diejenige von OVERMAN *et al.* sind wie folgt:

Formel von GAINES ····· Milcheiweiss(%) = 1.46 + 0.4 × Milchfett(%)
Formel von OVERMAN *et al.*···Milcheiweiss(%) = 1.1004 + 0.6531 × Michfett(%)
(für Holsteinmilch)

## (III) ERGEBNISSE UND BESPRECHUNG

Aus Tabelle 1 ist es ersichtlich, dass die Werte nach der Formel von GAINES beinahe immer kleiner sind als diejenigen nach der KJELDAHL-Methode, dass die Werte nach der Formel von OVERMAN *et al.* beinahe immer grösser sind als diejenigen nach der KJELDAHL-Methode und dass die Werte der Formoltitration denjenigen nach der KJELDAHL-Methode beinahe gleich sind.

Die Korrelationen zwischen den Werten nach GAINES, nach OVERMAN *et al.* bzw. nach der Formoltitration und denjenigen nach KJELDAHL wurden berechnet[23]. Zwischen dem Milcheiweissgehalt nach GAINES und demjenigen nach KJELDAHL ($r = +0.3728$) und zwischen dem Milcheiweissgehalt nach OVERMAN *et al.* und

Tabelle 1. Fettgehalt und Eiweissgehalt der Kuhmilch (%)

| Probe Nr. | Fettgehalt | Eiweissgehalt | | | |
|---|---|---|---|---|---|
| | | Nach der Formel von Gaines | Nach der Formel von Overman | Nach Formoltitration | Nach der Kjeldahlmethode |
| 1 | 3.30 | 2.78 | 3.26 | 3.06 | 3.07 |
| 2 | 3.10 | 2.70 | 3.13 | 2.98 | 2.96 |
| 3 | 3.10 | 2.70 | 3.13 | 3.01 | 2.98 |
| 4 | 2.30 | 2.38 | 2.60 | 2.98 | 2.96 |
| 5 | 3.40 | 2.82 | 3.32 | 2.94 | 2.95 |
| 6 | 3.70 | 2.94 | 3.52 | 3.09 | 3.13 |
| 7 | 3.50 | 2.86 | 3.39 | 2.98 | 3.00 |
| 8 | 3.10 | 2.70 | 3.13 | 3.01 | 3.07 |
| 9 | 3.60 | 2.90 | 3.45 | 2.89 | 2.94 |
| 10 | 3.10 | 2.70 | 3.13 | 2.99 | 3.03 |
| 11 | 4.10 | 3.10 | 3.78 | 3.03 | 3.07 |
| 12 | 3.70 | 2.94 | 3.52 | 2.98 | 2.96 |
| 13 | 3.50 | 2.86 | 3.39 | 2.89 | 2.96 |
| 14 | 3.40 | 2.82 | 3.32 | 2.92 | 2.96 |
| 15 | 3.30 | 2.78 | 3.26 | 2.89 | 2.95 |
| 16 | 2.40 | 2.42 | 2.67 | 2.98 | 3.02 |
| 17 | 3.25 | 2.76 | 3.22 | 2.79 | 2.83 |
| 18 | 3.90 | 3.02 | 3.65 | 3.06 | 3.05 |
| 19 | 3.40 | 2.82 | 3.32 | 2.94 | 2.92 |
| 20 | 3.25 | 2.76 | 3.22 | 2.81 | 2.84 |
| 21 | 3.55 | 2.88 | 3.42 | 3.06 | 3.04 |
| 22 | 3.50 | 2.86 | 3.39 | 3.13 | 3.06 |
| 23 | 4.20 | 3.14 | 3.84 | 3.21 | 3.28 |
| 24 | 3.40 | 2.82 | 3.32 | 3.15 | 3.11 |
| 25 | 3.35 | 2.80 | 3.29 | 2.98 | 2.98 |
| 26 | 3.30 | 2.78 | 3.26 | 2.84 | 2.81 |
| 27 | 3.30 | 2.78 | 3.26 | 3.04 | 3.05 |
| 28 | 3.30 | 2.78 | 3.26 | 2.81 | 2.77 |
| 29 | 3.20 | 2.74 | 3.19 | 2.86 | 2.80 |
| 30 | 3.20 | 2.74 | 3.19 | 2.84 | 2.81 |
| **Mittel** | — | **2.80** | **3.29** | **2.97** | **2.98** |

demjenigen nach KJELDAHL ($r = +0.3740$) wurden gesicherte, schwach positive Korrelationen errechnet. Dagegen bestand zwischen dem Milcheiweissgehalt nach Formoltitration und demjenigen nach KJELDAHL eine hoch gesicherte, straff positive Korrelation ($r = +0.9349$).

Unter Berücksichtigung der Tatsache, dass die Korrelation zwischen Fett- und Eiweissprozenten meistens niedrig und nicht gesichert ist und dass ferner die Ergebnisse der verschiedenen Forscher auf diesem Gebiet erheblich auseinandergehen, riet SALERNO[24], ausser den Fettprozenten der Kuhmilch auch die Eiweissprozente zu bestimmen, umso mehr als letztere mit genügender Genauigkeit und ohne be-

sondere Mühe durch die Formoltitration festgestellt werden können. Auch SOLBERG *et al.*[25] fanden eine grössere Korrelation zwischen dem Milcheiweissgehalt nach Formoltitration und demjenigen nach der KJELDAHL-Methode als zwischen dem Milchfettgehalt und dem Milcheiweissgehalt nach der KJELDAHL-Methode.

Unter Berücksichtigung der Resultate der verschiedenen Forscher und der jetzigen Untersuchungsergebnisse lässt sich die Schlussfolgerung ziehen, dass die Formoltitration als Eiweiss-Schnellbestimmung in der Molkereipraxis verwendet werden kann, aber dass die Formel von GAINES bzw die von OVERMAN *et al.* dafür nicht geeignet sind.

## (IV) ZUSAMMENFASSUNG

Zur Prüfung der Anwendbarkeit der Fomoltitration in der Molkereipraxis wurden die Werte der Formoltitration mit denjenigen nach der KJELDAHL-Methode verglichen. Auch die Werte nach der Formel von GAINES bzw. nach der von OVERMAN *et al.* wurden mit denjenigen nach der KJELDAHL-Methode verglichen.

Zwischen dem Milcheiweissgehalt nach Formoltitration und demjenigen nach der KJEDAHL-Methode besteht eine hoch gesicherte straffe positive Korrelation ($r = +0.9349$), dagegen zwischen dem Milcheiweissgehalt nach der Formel von GAINES und demjenigen nach der KJELDAHL-Methode ($r = +0.3728$) und zwischen dem Milcheiweissgehalt nach der Formel von OVERMAN *et al.* und demjenigen nach der KJELDAHL-Methode ($r = +0.3740$) bestehen gesicherte schwach positive Korrelationen.

In der Molkereipraxis kann die Formoltitration als Eiweiss-Schnellbestimmung verwendet werden, aber die Formel von GAINES bzw. die von OVERMAN *et al.* sind dafür nicht geeignet.

Wir danken herzlich Herrn H. HECKER für seine sehr freundliche und sorgfältige Berichtigung des Manuskriptes.

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# Jahreszeitliche Schwankungen des Gehaltes an Kasein und Molkeneiweiss der Kuhmilch

Yûzô Anagama und Takayasu Kami

*Institut für Tierzuchtlehre der Fakultät für Fischerei- u. Tierzuchtlehre an der Universität Hiroshima, Fukuyama, Japan*

(Tabellen 1—4)

## (I) EINLEITUNG

Über den jahreszeitlichen Einfluss auf die Höhe des Kasein- und Molkeneiweissgehaltes der Kuhmilch liegen nur spärliche Berichte vor. Neseni & Körprich[1] in Deutschland berichteten, dass die Kurve für den Kaseingehalt der Kuhmilch zwei Maxima in den Monaten Februar und Dezember und zwei Minima in den Monaten April und Juli aufwies und dass die Albumin-Globulin-Fraktion durch die Jahreszeit nicht so beträchtliche Schwankungen zeigte. Torre & Fontanella[2] in Italien fanden, dass der Kaseingehalt der Kuhmilch im November mit 2.58 % am höchsten und im April mit 2.41 % am niedrigsten war und dass auch der Laktalbumingehalt eine ähnliche Schwankung zeigte. Nach Waite *et al.*[3] in Schottland erreichte der Kaseingehalt den höchsten Punkt im Mai/Juni und wieder im September und den niedrigsten vom Januar bis März.

Wir[4][5] fanden, dass die Erniedrigung des Gehaltes an fettfreier Trockenmasse der Kuhmilch im Sommer im Fukuyama-Distrikt (Westjapan) durch Verminderung des Eiweissgehaltes und des Milchzuckergehaltes verursacht wurde, wobei jener die wichtigere Rolle spielte. Auf welcher Fraktion des Milcheiweisses beruht nun die Verminderung des Gehaltes an Milcheiweiss ? Die vorliegende Arbeit berichtet über die Ergebnisse der Untersuchung dieses Problems.

## (II) MATERIAL UND METHODE

Die Untersuchung wurde ausgeführt an gemischten Milchproben, welche aus den 3 Molkereien der Stadt Fukuyama in Hiroshima-ken in je 5 Tagen von jeder Molkerei monatlich während eines Jahres gewonnen wurden. Die Milchproben wurden in jeder Molkerei aus einem Pasteurisationsapparat von etwa 360 *l* Inhalt vor Erhitzung entnommen. Jeder Molkerei wurde täglich Milch von ca. 280 Milchkühen (Holsteinrasse) geliefert.

Die Untersuchung wurde in der Zeit von Mitte November 1958 bis Ende Oktober 1959 durchgeführt.

Die Gesamteiweiss- und Molkeneiweissbestimmung erfolgte nach Rowland[6]. Der Kaseingehalt wurde aus den Werten des Gesamteiweissgehaltes und des Molkeneiweissgehaltes berechnet.

## (III) ERGEBNISSE UND BESPRECHUNG

Tabelle 1. Eiweissgehalt der Kuhmilch im Verlaufe des Jahres (%)

| Monat \ Probe Nr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Nov. '58 | 2.86 | 3.16 | 2.96 | 3.01 | 3.09 | 2.96 | 2.97 | 2.92 |
| Dez. | 2.81 | 2.91 | 3.16 | 2.93 | 2.98 | 2.85 | 2.87 | 2.94 |
| Jan. '59 | 2.81 | 3.09 | 2.85 | 2.93 | 3.16 | 2.98 | 2.89 | 2.89 |
| Feb. | 2.95 | 2.71 | 2.94 | 2.92 | 3.05 | 2.83 | 2.90 | 2.85 |
| März | 2.87 | 2.86 | 2.94 | 2.71 | 2.83 | 2.83 | 2.87 | 2.93 |
| Apr. | 2.89 | 2.86 | 2.91 | 2.90 | 2.88 | 2.74 | 2.86 | 2.84 |
| Mai | 2.91 | 2.90 | 2.86 | 2.83 | 2.87 | 2.89 | 2.88 | 2.70 |
| Juni | 2.81 | 2.78 | 2.69 | 2.78 | 2.78 | 2.82 | 2.74 | 2.78 |
| Juli | 2.77 | 2.79 | 2.74 | 2.77 | 2.73 | 2.75 | 2.72 | 2.71 |
| Aug. | 2.80 | 2.85 | 2.84 | 2.97 | 2.70 | 2.76 | 2.79 | 2.67 |
| Sep. | 2.93 | 2.83 | 2.81 | 2.87 | 2.87 | 2.70 | 2.81 | 2.93 |
| Okt. | 3.01 | 2.99 | 2.94 | 2.95 | 2.93 | 2.94 | 2.97 | 3.03 |

| Monat \ Probe Nr. | 9 | 10 | 11 | 12 | 13 | 14 | 15 | **Mittel** |
|---|---|---|---|---|---|---|---|---|
| Nov. '58 | 2.95 | 2.92 | 2.87 | 2.88 | 2.75 | 2.93 | 2.85 | **2.94** |
| Dez. | 2.87 | 2.91 | 3.01 | 2.86 | 2.82 | 2.79 | 2.88 | **2.91** |
| Jan. '59 | 2.81 | 2.64 | 2.88 | 2.99 | 2.94 | 2.71 | 2.70 | **2.88** |
| Feb. | 2.86 | 2.82 | 2.67 | 2.88 | 2.88 | 2.93 | 2.87 | **2.87** |
| März | 2.96 | 2.98 | 2.72 | 2.77 | 2.85 | 2.88 | 2.93 | **2.86** |
| Apr. | 2.77 | 2.85 | 2.84 | 2.88 | 2.86 | 2.88 | 2.81 | **2.85** |
| Mai | 2.84 | 2.78 | 2.77 | 2.77 | 2.72 | 2.73 | 2.77 | **2.81** |
| Juni | 2.80 | 2.75 | 2.70 | 2.66 | 2.75 | 2.63 | 2.71 | **2.75** |
| Juli | 2.79 | 2.74 | 2.69 | 2.77 | 2.71 | 2.77 | 2.72 | **2.74** |
| Aug. | 2.81 | 2.83 | 2.78 | 2.76 | 2.78 | 2.77 | 2.78 | **2.79** |
| Sep. | 2.85 | 2.84 | 2.86 | 2.91 | 2.83 | 2.91 | 2.83 | **2.85** |
| Okt. | 2.90 | 2.96 | 2.95 | 2.94 | 2.84 | 2.89 | 2.85 | **2.94** |

Tabelle 1 zeigt, dass der Gesamteiweissgehalt der Kuhmilch im Oktober/November mit 2.94% am höchsten und im Juli mit 2.74% am niedrigsten ist. Der Gesamteiweissgehalt der Kuhmilch im Oktober/November ist im Durchschnitt beinahe gleich dem durchschnittlichen Gehalt im Dezember, Januar oder Februar, aber er ist bedeutend höher als die durchschnittlichen Gehalte anderer Kalendermonate. Und der Gesamteiweissgehalt der Kuhmilch im Juli ist im Durchschnitt beinahe gleich dem durchschnittlichen Gehalt im Juni, aber er ist bedeutend niedriger als die durchschnittlichen Gehalte anderer Kalendermonate. Dieses Untersuchungsergebnis stimmt mit denjenigen von uns[5] aus den Jahren 1957/58 und von OVERMAN[7] in Staat Illinois in USA ziemlich gut überein.

Aus Tabelle 2 geht hervor, dass der Molkeneiweissgehalt der Kuhmilch von der Jahreszeit nicht so beträchtlich beeinflusst wird, dass aber der Gehalt vom Juni bis September sich etwas vermindert. Dieses Untersuchungsergebnis

stimmt mit demjenigen von NESENI & KÖRPRICH[1] in Deutschland ziemlich gut überein.

Tabelle 2. Molkeneiwissgehalt der Kuhmilch im Verlaufe des Jahres (%)

| Monat \ Probe Nr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Nov. '58 | 0.66 | 0.68 | 0.63 | 0.64 | 0.65 | 0.66 | 0.69 | 0.69 |
| Dez. | 0.62 | 0.63 | 0.66 | 0.65 | 0.66 | 0.67 | 0.68 | 0.66 |
| Jan. '59 | 0.66 | 0.70 | 0.66 | 0.68 | 0.69 | 0.65 | 0.66 | 0.67 |
| Feb. | 0.67 | 0.62 | 0.67 | 0.67 | 0.68 | 0.67 | 0·66 | 0.67 |
| März | 0.69 | 0.67 | 0.66 | 0.62 | 0.66 | 0.66 | 0.67 | 0.66 |
| Apr. | 0.66 | 0.65 | 0.68 | 0.68 | 0.66 | 0.65 | 0.66 | 0.66 |
| Mai | 0.65 | 0.65 | 0.65 | 0.65 | 0.66 | 0.69 | 0.68 | 0.67 |
| Juni | 0.63 | 0.63 | 0.61 | 0.62 | 0.62 | 0.67 | 0.65 | 0.67 |
| Juli | 0.61 | 0.64 | 0.62 | 0.62 | 0.61 | 0.64 | 0.64 | 0.62 |
| Aug. | 0.60 | 0.62 | 0.63 | 0.61 | 0.59 | 0.62 | 0.64 | 0.63 |
| Sep. | 0.63 | 0.60 | 0.61 | 0.60 | 0.61 | 0.62 | 0.62 | 0.65 |
| Okt. | 0.66 | 0.67 | 0.65 | 0.64 | 0.66 | 0.65 | 0.67 | 0.71 |

| Monat \ Probe Nr. | 9 | 10 | 11 | 12 | 13 | 14 | 15 | **Mittel** |
|---|---|---|---|---|---|---|---|---|
| Nov. '58 | 0.72 | 0.70 | 0.62 | 0.62 | 0.62 | 0.62 | 0.64 | **0.66** |
| Dez. | 0.69 | 0.67 | 0.67 | 0.66 | 0.65 | 0.66 | 0.64 | **0.66** |
| Jan. '59 | 0.65 | 0.66 | 0.64 | 0.65 | 0.65 | 0.64 | 0.66 | **0.66** |
| Feb. | 0.68 | 0.66 | 0.62 | 0.65 | 0.67 | 0.64 | 0.65 | **0.66** |
| März | 0.70 | 0.68 | 0.62 | 0.67 | 0.66 | 0.67 | 0.66 | **0.66** |
| Apr. | 0.65 | 0.66 | 0.64 | 0.67 | 0.64 | 0.66 | 0.65 | **0.66** |
| Mai | 0.64 | 0.66 | 0.64 | 0.62 | 0.60 | 0.60 | 0.62 | **0.64** |
| Juni | 0.65 | 0.65 | 0.58 | 0.60 | 0.59 | 0.61 | 0.62 | **0.63** |
| Juli | 0.68 | 0.64 | 0.67 | 0.64 | 0.63 | 0.63 | 0.62 | **0.63** |
| Aug. | 0.66 | 0.62 | 0.61 | 0.61 | 0.62 | 0.62 | 0.62 | **0.62** |
| Sep. | 0.62 | 0.62 | 0.65 | 0.64 | 0.63 | 0.64 | 0.64 | **0.62** |
| Okt. | 0.64 | 0.66 | 0.64 | 0.63 | 0.64 | 0.64 | 0.65 | **0.65** |

Aus Tabelle 3 ist es ersichtlich, dass der Kaseingehalt der Kuhmilch im Oktober mit 2.29 % am höchsten und im Juli mit 2.11 % am niedrigsten ist. Der Kaseingehalt im Oktober ist im Durchschnitt beinahe gleich dem durchschnittlichen Gehalt im November oder Dezember, aber er ist bedeutend höher als die durchschnittlichen Gehalte anderer Kalendermonate. Und der Kaseingehalt im Juli ist im Durchschnitt beinahe gleich dem durchschnittlichen Gehalt im Juni, aber er ist bedeutend niedriger als die durchschnittlichen Gehalte anderer Kalendermonate. Dieses Untersuchungsergebnis stimmt nicht mit denjenigen der Autoren in Europa überein.

Auf diese Weise fanden wir, dass der Gesamteiweiss- und Kaseingehalt der Kuhmilch im Juni und Juli sich erheblich verminderte und dass der Molkeneiweissgehalt vom Juni bis September sich etwas verminderte. Daher könnte man

Tabelle 3. Kaseingehalt der Kuhmilch im Verlaufe des Jahres (%)

| Monat \ Probe Nr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Nov. '58 | 2.20 | 2.48 | 2.33 | 2.37 | 2.44 | 2.30 | 2.28 | 2.23 |
| Dez. | 2.19 | 2.28 | 2.50 | 2.28 | 2.32 | 2.18 | 2.19 | 2.28 |
| Jan. '59 | 2.15 | 2.39 | 2.19 | 2.25 | 2.47 | 2.33 | 2.23 | 2.22 |
| Feb. | 2.28 | 2.09 | 2.27 | 2.25 | 2.37 | 2.16 | 2.24 | 2.18 |
| März | 2.18 | 2.19 | 2.28 | 2.09 | 2.17 | 2.17 | 2.20 | 2.27 |
| Apr. | 2.23 | 2.21 | 2.23 | 2.22 | 2.22 | 2.09 | 2.20 | 2.18 |
| Mai | 2.26 | 2.25 | 2.21 | 2.18 | 2.21 | 2.20 | 2.20 | 2.03 |
| Juni | 2.18 | 2.15 | 2.08 | 2.16 | 2.16 | 2.15 | 2.09 | 2.11 |
| Juli | 2.16 | 2.15 | 2.12 | 2.15 | 2.12 | 2.11 | 2.08 | 2.09 |
| Aug. | 2.20 | 2.23 | 2.21 | 2.36 | 2.11 | 2.14 | 2.15 | 2.04 |
| Sep. | 2.30 | 2.23 | 2.20 | 2.27 | 2.26 | 2.08 | 2.19 | 2.28 |
| Okt. | 2.35 | 2.32 | 2.29 | 2.31 | 2.27 | 2.29 | 2.30 | 2.32 |

| Monat \ Probe Nr. | 9 | 10 | 11 | 12 | 13 | 14 | 15 | **Mittel** |
|---|---|---|---|---|---|---|---|---|
| Nov. '58 | 2.23 | 2.22 | 2.25 | 2.26 | 2.13 | 2.31 | 2.21 | **2.28** |
| Dez. | 2.18 | 2.24 | 2.34 | 2.20 | 2.17 | 2.13 | 2.24 | **2.25** |
| Jan. '59 | 2.16 | 1.98 | 2.24 | 2.34 | 2.29 | 2.07 | 2.04 | **2.22** |
| Feb. | 2.18 | 2.16 | 2.05 | 2.23 | 2.21 | 2.29 | 2.22 | **2.21** |
| März | 2.26 | 2.30 | 2.10 | 2.10 | 2.19 | 2.21 | 2.27 | **2.20** |
| Apr. | 2.12 | 2.19 | 2.20 | 2.21 | 2.22 | 2.22 | 2.16 | **2.19** |
| Mai | 2.20 | 2.12 | 2.13 | 2.15 | 2.12 | 2.13 | 2.15 | **2.17** |
| Juni | 2.15 | 2.10 | 2.12 | 2.06 | 2.16 | 2.02 | 2.09 | **2.12** |
| Juli | 2.11 | 2.10 | 2.02 | 2.13 | 2.08 | 2.14 | 2.10 | **2.11** |
| Aug. | 2.15 | 2.21 | 2.17 | 2.15 | 2.16 | 2.15 | 2.16 | **2.17** |
| Sep. | 2.23 | 2.22 | 2.21 | 2.27 | 2.20 | 2.27 | 2.19 | **2.23** |
| Okt. | 2.26 | 2.30 | 2.31 | 2.31 | 2.20 | 2.25 | 2.20 | **2.29** |

in diesem Zusammenhang auf die Vermutung kommen, dass die Senkung des Gesamteiweissgehaltes der Kuhmilch im Sommer durch Verminderung des Kaseingehaltes verursacht wurde, wobei die Verminderung des Molkeneiweissgehaltes eine kleine Rolle spielte.

In früherer Untersuchung fanden wir[5], dass die Erniedrigung des Gehaltes an fettfreier Trockenmasse der Kuhmilch im Sommer im Fukuyama-Distrikt (Westjapan) durch Verminderung des Milcheiweissgehaltes und des Milchzuckergehaltes verursacht wurde, wobei jener die wichtigere Rolle spielte. Daher könnte man auf die Schlussfolgerung kommen, dass die Erniedrigung des Gehaltes an fettfreier Trockenmasse der Kuhmilch im Sommer im Distrikt grösstenteils durch Verminderung des Kaseingehaltes verursacht wurde, wobei auch die Senkung des Milchzucker- und Molkeneiweissgehaltes eine kleine Rolle spielte.

Worauf ist es nun zurückzuführen, dass der Kasein- und Molkeneiweissgehalt im Sommer sich vermindern? RIDDET *et al.*[8,9], ROWLAND[10,11], HOLMES *et al.*[12,13], WAITE[14], WAITE *et al.*[3], PATCHELL[15], EDWARDS[16] sowie COMBERG & VOIGT-

LÄNDER[17] betrachteten die Verminderung des Milcheiweissgehaltes als das Resultat der Unterernährung der Milchkühe. Dagegen berichteten GRIFFITHS & FEATHERSTONE[18], dass keine bedeutende Rückwirkung der Kühe durch Extrafütterung von Kalorien erhalten wurde, dass die Kühe nicht von unmässiger Mastitis befallen wurden und dass die hauptsächliche Ursache der Verminderung des Gehaltes an fettfreier Trockenmasse in ihrem Versuch genetisch war. Auch BURT[19] fand, dass die Verminderung des Gehaltes an fettfreier Trockenmasse der Milch bei Kühen, welche gut gefüttert und gehalten wurden, stattfand und dass diese Verhältnisse auf genetische oder unbekannte Faktoren zurückzuführen waren.

DAVIS *et al.*[20] berichteten, dass die Verminderung des Gehaltes an fettfreier Trockenmasse der Milch im Sommer vielleicht hauptsächlich durch die hohe Lufttemperatur verursacht werden möchte. Tatsächlich fanden REGAN & RICHARDSON[21], dass die hohe Lufttemperatur über 80° F (ca. 27°C) das Kasein in der Milch verringerte. Ebenso fanden COBBLE & RAGSDALE[22] sowie COBBLE & HERMAN[23], dass die hohe Lufttemperatur über 80～90°F das Eiweiss in der Milch verringerte. TANEJA[24] berichtete, dass Rinder den Schweiss brauchen, um ein Steigen der Körpertemperatur zu verhüten, aber dass die Menge des abgesonderten Schweisses nicht genug ist, um bei hohen Lufttemperaturen die Thermoneutralität der Rinder zu erhalten.

Es wird vielleicht hauptsächlich auf die hohe Lufttemperatur mit hoher Luftfeuchtigkeit des Sommers im Fukuyama-Distrikt zurückzuführen sein, dass in diesem Falle der Kasein- und Molkeneiweissgehalt der Kuhmilch im Sommer sich verminderte (vgl. Tabelle 4). Aber auch Unterernährung der Kühe und unbekannte Faktoren mögen Einfluss haben. Für eine genaue Erklärung dieser Verhältnisse sind jedoch noch andere weitere Untersuchungen erforderlich.

Tabelle 4. Lufttemperatur und Luftfeuchtigkeit im Fukuyama-Distrikt

| Monat | Nov. 1958 | Dez. | Jan. 1959 | Feb. | März | Apr. | Mai | Juni | Juli | Aug. | Sep. | Okt. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lufttemperatur (°C) | | | | | | | | | | | | |
| Min. | 1.2 | −1.5 | −6.5 | −3.8 | −2.9 | 3.2 | 7.9 | 11.2 | 18.9 | 17.9 | 12.5 | 6.1 |
| Max. | 23.2 | 16.8 | 15.6 | 16.2 | 19.7 | 24.4 | 28.6 | 32.4 | 34.3 | 36.7 | 34.3 | 27.2 |
| Mittel | 11.3 | 7.6 | 3.5 | 7.1 | 8.5 | 13.7 | 17.9 | 22.3 | 26.3 | 27.0 | 24.1 | 17.3 |
| Luftfeuchtigkeit (%) | | | | | | | | | | | | |
| Mittel | 76 | 75 | 73 | 79 | 71 | 72 | 76 | 72 | 83 | 80 | 77 | 77 |

(Nach den Messungen der Wetterwarte Matsunaga)

## (IV) ZUSAMMENFASSUUNG

(1) Der Gesamteiweiss-, Molkeneiweiss- und Kaseingehalt der Kuhmilch im Fukuyama-Distrikt (Westjapan) wurde während eines Jahres bestimmt.

(2) Der Gesamteiweissgehalt der Milch war im Oktober/November mit 2.94

% am höchsten und im Juli mit 2.74 % am niedrigsten.

(3) Der Molkeneiweissgehalt der Milch wurde von der Jahreszeit nicht so beträchtlich beeinflusst, verminderte sich jedoch etwas vom Juni bis September.

(4) Der Kaseingehalt der Milch war im Oktober mit 2.29 % am höchsten und im Juli mit 2.11 % am niedrigsten.

(5) Es ist zu vermuten, dass die Erniedrigung des Gehaltes an fettfreier Trockenmasse der Milch im Sommer in diesem Distrikt hauptsächlich durch Verminderung des Kaseingehaltes verursacht wurde, wobei auch die Senkung des Milchzucker- und Molkeneiweissgehaltes eine kleine Rolle spielte, und dass es vielleicht auf die hohe Lufttemperatur mit hoher Luftfeuchtigkeit des Sommers in diesem Distrikt zurückzuführen sein wird.

Wir danken herzlich Herrn H. Hecker für seine sehr freundliche und sorgfältige Berichtigung des Manuskriptes.

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# A Single Differential Staining Method for Vaginal Smears

Shunsaku FUJII

*Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama*

(Plate 1)

Since the observation of STOCKARD and PAPANIKOLAOU (1917) on the cyclic cytological changes of vaginal smears in guineapigs in accordance with their estrous cycle, many morphological studies have been made on vaginal smears of different mammals by many investigators. Recently, the examination of vaginal smears has come to be significant not only in the determination of each stage of the estrous cycle and in sexual hormone tests, but also in the clinical use. As for the staining of vaginal smears, there are many methods using such a single stain as methylene blue and Giemsa stain or such a compound stain as Masson's trichrome and Heidenhain's azan stain. Especially, SHORR (1940a, 1940b, 1941), PAPANIKOLAOU (1942), and CUYLER (1932) have reported a single differential staining method which is generally used for vaginal smears. The principle of this method of staining consits in the demonstration of changes in stainability of epithelial cells by using a staining solution composed of some acidic dyes. For instance, orange G, scarlet red, and fast green are used in Shorr's method, by which cornified cells are stained blilliant orange-red and non-cornified cells blue. By Papanikolaou's method, in which light green, Bismarck brown, and eosin Y are used, acidophilic cells are stained red to orange and basophilic cells green or greenish blue.

The method presented in this paper is essentially the same as the methods of the above-mentioned authors. In this method are used three kinds of acidic dyes which differ in dispersity and color-tone: picric acid, aniline blue, and ponceau 2R. This method is based especially upon the theory of MÖLLENDORFF (1924) and SEKI (1936, 1954) on "the ultrastructural density of tissues and its stainability". It is very simple and brings about a rapid differentiation for vaginal smears. So it will be useful for rapid inspection of vaginal smears.

## (I) STAINING METHOD

1. Vaginal smear is sucked up by a small pipette, with a rubber cap, containing a small amount of physiological salt solution which is used for washing the surface of the vaginal mucous membrane. It is placed on a slide glass and spread out thinly with tip of the pipette.

2. The slide, when almost dried, is fixed with 10 per cent formalin solution

for about 10 to 15 minutes.

3. It is rinsed with running tap water for about 10 minutes.

4. The slide glass is pressed lightly with filter paper to remove the excessive water on it and stained with the staining solution mentioned below for about 10 minutes on the thermostat at about 37°C, being wavered occasionally.

The staining solution is prepared as follows: 0.5 per cent aqueous solution of aniline blue, picric acid, and ponceau 2R are mixed at the rate of 1 : 3 : 3, respectively. Then glacial acetic acid is added to the whole amount of the mixture at the rate of 1.5 per cent. For aniline blue Merck's product is used and for ponceau 2R and picric acid Katayama's products are employed.

It is preferable to use the staining solution a few days after preparation than immediately after preparation. The solution can be preserved for several months when well stoppered.

5. The slide may be rinsed for a very short time. However, rinsing may be skipped. Prolonged washing is undesirable, since a yellow and blue tone of the preparation is removed.

6. It is carried directly into 90 per cent alcohol, dehydrated through absolute alcohol, and made transparent in xylol. Ten dippings may be enough in each of the alcohol.

In preparing a smear slide, it is most important not to allow the smear to dry excessively before fixation, since drying alters the staining properties of the cells contained in the smear. In any case, moreover, drying is undesirable during the whole process of staining. The thickness of the smear has influence also on the stainability of the cells. Therefore, care must be taken of the secretion of the estrous stage which is rich in cell elements and liable to flock into masses. An excellent picture stained is shown in an area which is poor in cell elements in the center of a smeared specimen. When many smears are examined simultaneously on the same slide glass, it is convenient to place one or two drops of fixative, with a pipette, on each of the smears as they are collected to prevent them from excessive drying. Then the slide with smears on it is finally put in the fixing bottle. The specimen must be examined immediately, as it is decolorized gradually.

## (II) RESULTS OF STAINING

This study was performed on the vaginal smears of the mouse. When the slide stained by this method is placed on a piece of white paper, it is possible to determine macroscopically from the specific tint of its staining color to what stage of the estrous cycle it belongs. Precise microscopic observation reveals a sharp difference in color tone between cornified and non-cornified cells. In the so-called cornified cells, which are large in size, containig no visible nuclei, the cell body is stained in a yellow tint homogeneously. In the non-cornified cells, which have nuclei, the cytoplasm takes various color tones, such as reddish-yellow, red, reddish-purple, purple, blue and the nuclei are stained yellowish-red slightly, as shown

in the figures attached. Among the other constituents of the smears, the leucocytes are stained blue or, sometimes, reddish-blue and mucus blue.

Fig. 1 shows the vaginal smears of a mouse in the diestrous stage. A large number of leucocytes stained in a blue color and a small number of younger nucleated cells are presented. Either cornified or pre-cornified cells in the process of cornification are hardly found.

Fig. 2 shows a picture of the proestrous stage. Leucocytes are decreased in number and large-sized, nucleated cells stained in various color tones increased in number. A few cornified cells are seen.

Fig. 3 shows a picture of the estrous stage. Only cornified cells stained in a yellow color are presented.

Fig. 4 shows a picture of the metaestrous stage. Leucocytes and nucleated cells in various color tones are seen. A few cornified cells still remain.

In these figures, it should be noted that the cytoplasm is stained yellow in the cornified cell and in various color tones in the non-cornified cell. Those findings indicate that the degree of cornification is different between the two kinds of cells. Such difference in staining color is explained as follows: according to SEKI, picric acid producing a yellow tint is of the highest dispersity, aniline blue producing a blue tint is of the lowest, and ponceau 2R producing a red tint is of moderate dispersity. In accordance with the advance in the process of cornification of cells, the ultrastructure of cell elements becomes dense gradually. Consequently, the denser the ultrastructure of a cell, the more stainable the cell may be with such small-moleculed dye as picric acid. That is, the more cornified cells are present, the more remarkable the yellowish tone is. It has been already confirmed by HINO (1948) in guineapigs with the azan staining method that the ultrastructure of epithelial cells in vaginal smears becomes dense with the progress of cornification.

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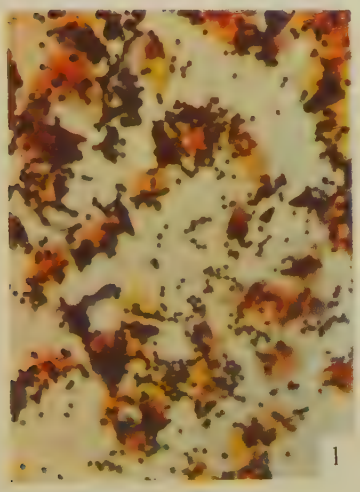
## EXPLANATION OF PLATE

Fig. 1. The vaginal smears of a mouse in the diestrous stage. ×150.
Fig. 2. The vaginal smears in the proestrous stage. ×150.
Fig. 3. The vaginal smears in the estrous stage. ×150.
Fig. 4. The vaginal smears in the metaestrous stage. ×150.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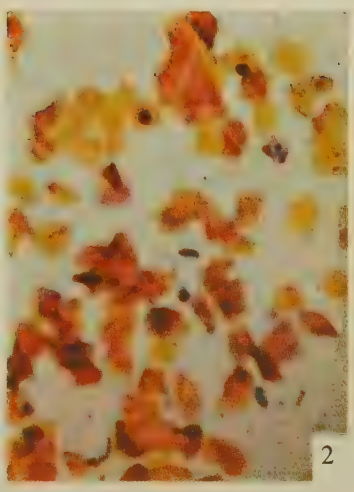
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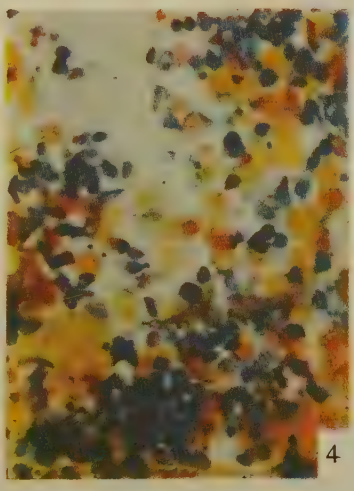
4

FUJII: Staining Method for Vaginal Smears

# The Growth of the Fish Otolith.

Takaaki IRIE

*Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama*

(Plates 1—4; Text-figs. 1—8; Tables 1—5)

## (I) INTRODUCTION

The sacculus otolith of the fish, together with its scale, is closely connected with the age and the growth of the fish. Most of the studies on the age and the growth of the fish have been carried out usually by means of observation of the zones in the scale because the observation of them is easier than those in the otolith. But, the zones in the scale are more obscure generally than those in the otolith. It is needless to say, therefore, that the studies can be carried out more reliably by means of the observation of the zones in the otolith than those in the scale. The effective factors of the zone formation of the fish otolith have been examined by some investigators,[1)—6)] yet, no accepted or established view on the factor has been attained until now. Accordingly, concerning the age of the fish and its growth history, it is difficult to expect accurate studies on them, and, therefore the number of the studies is actually few.

This is the reason why the study on the fish otolith had been begun by the present writer to discover a procedure for the estimation of the individual growth history of the fish through its zone. The writer already examined the otolith of the fish caught in various seasons by fishing, and the crystal texture and the forming season of the zones were thus made clear.[7)—8)]

In the present study, the otolith in the marine teleosts reared mostly by the writer, not caught from the sea, were directly examined through the year by means of electron microscope, X-rays and radioactive isotope $Ca^{45}$. Consequently, the formation of the opaque and translucent zones of the otolith and the various factors concerning its formation are here to be elucidated.

## (II) MATERIALS

The materials used in the present study are marine teleosts, Fish-A, $B_1$, $B_2$, $B_3$, $C_1$ and $C_2$ as are shown in Table 1. They were originally caught in the Inland Sea of Seto (Seto-naikai), and, except for Fish-A, they were reared in the running sea water, and were given enough fish meat as their food once a day till they were used in the following experiments since they were caught. The fish belonging to Fish-$B_2$ were at least more than one year old when used in the experiment because they were reared by the present writer for more than 10 months.

Table 1. The various marine teleosts used in this study. The body length and the age indicate those at the period in which the fish were caught.

| | Scientific name | Japanese name | Time caugth | Body length | Age | Object & Instrument |
|---|---|---|---|---|---|---|
| Fish-A | *Argyrosomus argentatus* (HOUTTUYN) | SHIROGUCHI | October, 1959 | 12~22 cm. | 2 | The crystal texture by using X-rays. |
| Fish-$B_1$ | *Lateolabrax japonicus* (CUVIER) | SUZUKI | April, 1957 | about 3 cm. | 0 | Effects of the food by using $Ca^{45}$. |
| Fish-$B_2$ | same as above | --- | April, 1958 | about 3 cm. | 0 | The crystal texture by using electron microscope. Effects of the concentration of water by using $Ca^{45}$. |
| Fish-$B_3$ | same as above | --- | April, 1959 | about 3 cm. | 0 | Formation of $CaCO_3$ by using $Ca^{45}$. |
| Fish-$C_1$ | *Mylio macrocephalus* (BASILEWSKY) | KURODAI | August, 1957 | about 7 cm. | 0 | Effects of the concentration of water by using $Ca^{45}$. |
| Fish-$C_2$ | same as above | --- | August, 1958 | about 7 cm. | 0 | Effects of the water temperature by using $Ca^{45}$. |

## (III) EXPERIMENTAL PROCEDURE

*Experiment-1* : Observation of the otolith slice by means of X-rays.

This observation was performed to make clear the status of micro-crystal grains of the calcium carbonate ($CaCO_3$) which is the main constituent of the otolith. As its specimens, the otoliths (8~11mm. in length, 6~8mm. in width, 3~4mm. in thickness) obtained from Fish-A (shown in Table 1) were used.

Each otolith was polished into a thin slice (0.1mm. thick) so as to make the surface plane perpendicular to the lengthwise axis of the otolith and to make it cross the center (shown in Fig. 1 and concerning this procedure confer the previous experiment.[7]). Laue photographs of every portion in this slice were taken by using X-rays through a narrow circular slit (0.09 mm. in diameter) emitted from

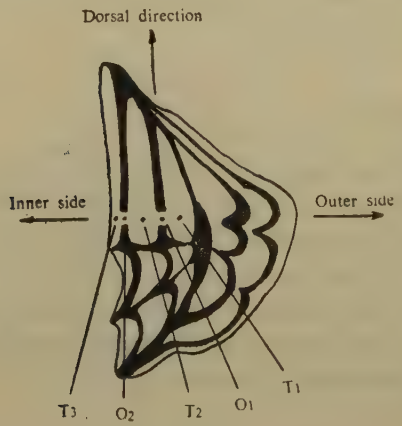


Fig. 1. Cross sectional patterns of the otolith of *Argyrosomus* (Japanese name: SHIROGUCHI, two age, body length: 20.5 cm., Body weight: 200g. Fish-A). The sectional plane is perpendicular to the lengthwise axis of the otolith and crosses its center. $T_1$, $T_2$, and $T_3$ are in the first, second and third translucent zones, and $O_1$ and $O_2$ are in the first and second opaque zones respectively.

a micro-focus ($0.01 \times 0.02\,mm.^2$) on the Cu-target. In this case, the beam of X-rays strikes a plane of the slice at right angle, and the photographic film was set perpendicularly to the incident beam behind the slice at the distance of 30 mm.

*Experiment-2*: Electron microscopical observation of the surface of otolith.

The surface of the otoliths which were taken out from Fish-$B_2$ (shown in Table 1) in February, May, July, August, September and December, 1959, was observed by means of electron microscope in the following way. Each otolith was washed carefully not to injure its surface and then dried in a room. Triafol seat (0.04 mm. in thickness) was adhered closely to its surface using aceton and was stripped off after a few minutes. Then Cr-preshadowed carbon replica was made from this triafol replica. This Cr-preshadowing was done from its lengthwise direction and its inclination to the surface was about 45°. This replica was divided into three divisions, Po., M. and An. as is indicated in Fig. 2-b. These replica was observed by means of AKASHI I. R. S.-50 Electron Microscope.

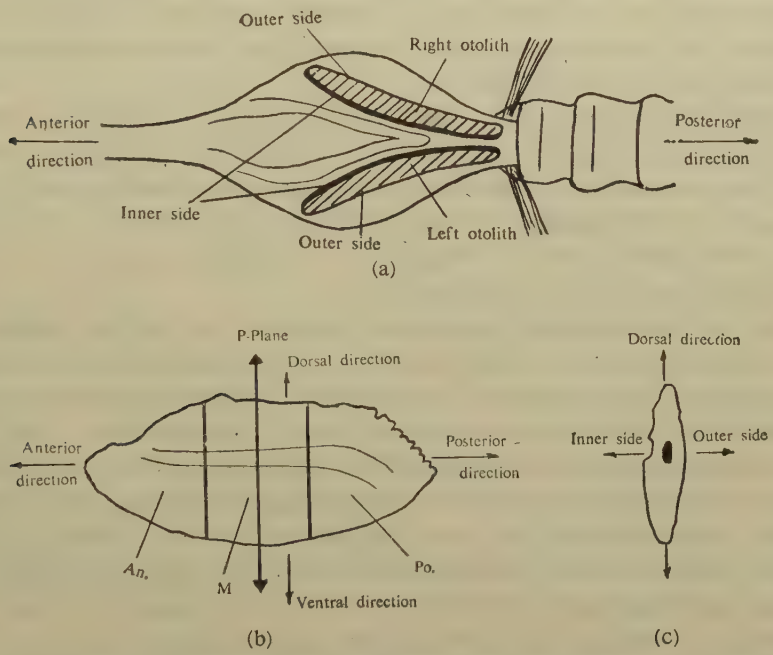


Fig. 2. Otolith of *Lateolabrax* (Japanese name: SUZUKI, Fish-$B_1$, $B_2$ & $B_3$).
(a) View from the ventral direction.
(b) The inner side surface of the otolith. P-Plane is perpendicular to the lengthwise direction of the otolith.
(c) The cross section of the P-Plane perpendicular to lengthwise direction of the otolith.

*Experiment-3*: Observation of the otolith slice by means of autoradiograph using $Ca^{45}$.

In order to make clear the growing direction of the otolith, its velocity and

the depositing location of calcium, this observation was done by means of autoradiograph using Fish-$B_3$ (shown in Table 1).

In June, 1959, similar sized fish taken out from the group of Fish-$B_3$ (grown up to about 6 cm.) were put into the rearing tanks at the rate of 3 fish per 30 *l.* of the sea water, and then the preparatory rearing of these fish was carried on for the following 5 days. When the preparatory rearing was concluded, radioactive isotope $Ca^{45}$ ($CaCl_2$ in HCl solution) was added at the rate of 4 $\mu$c. per *l.* of the water. Now, the amount of this $Ca^{45}$ was determined through many preparatory experiments in the previous year. These fish were reared continually in the water containing $Ca^{45}$ for the following 10 days. Half of the water in the tank was exchanged every other day for the fresh sea water which has the concentration of 18‰ in chlorinity, its temperature, 21～25°C and saturating degree of disolved oxygen, above 95%. The amount of $CaCl_2$ added together with $Ca^{45}$ $Cl_2$ was so small that no significant variation was discovered concerning the pH, the concentration and the amount of calcium. Fish meat was given for their food sufficiently once a day and they took it with positive appetite. When this way of rearing using $Ca^{45}$ was concluded, these fish were put into the running sea water, and were reared with the same food.

In the following September, these fish grown up to about 12 cm. were put into the water containing $Ca^{45}$ and were reared again by the procedure similar to that in June. In September, however, water temperature was kept at 20～23°C and 2.7 $\mu$c./*l.* of $Ca^{45}$ was used in order to keep the radioactivity at the same intensity as the previous one, because 4 $\mu$c./*l.* of radioactivity used in June is to decay naturally to 2.7 $\mu$c./*l.* in September.

When the second rearing experiment using $Ca^{45}$ was finished, otoliths were taken out from these fish. Each otolith was polished into a thin slice (about 0.2 mm. thick) so as to make the surface plane (P-Plane indicated in Fig. 2-b) perpendicular to its lengthwise axis and to make it cross the center. One side of this slice was adhered closely to a glass plate with wax, and its micro-autoradiograph was prepared by means of the stripping-emulsion method. Then emulsion membrane was stripped off from its surface and was fixed on another glass plate.

By the same procedure as is described above, micro-autoradiographs of the otolith slice were also made using other fish, which were reared for 10 days twice, September and Novèmber, in the rearing tank containing $Ca^{45}$.

*Experiment-4*: Effect of the concentration of sea water concerning the growth of otolith.

Amount of calcium taken from the sea water and deposited on the otolith surface was examined by using $Ca^{45}$ as a tracer. Nine fish of similar size were taken out from the group of Fish-$C_1$ (shown in Table 1), and were divided into three, A-, B- and C-group. Each group which has three fish was put into a rearing tank having 30 *l.* of sea water. In the first 5 days, the rearing water of A-group was diluted gradually to 17‰ in chlorinity, B-group to 14‰ and C-group to 10‰ respectively using fresh water. During the following 5 days, preparatory rearing

of these fish was carried on in the tank keeping each concentration unchanged. During this preparatory rearing, the enough fish meat was given as their food once a day.

When this preparatory rearing was concluded, radioactive isotope $Ca^{45}$ ($CaCl_2$ in HCl solution) was added to every group at the rate of 3 $\mu$c. per *l.* of the sea water. The amount of $CaCl_2$ added together with $Ca^{45}Cl_2$ was so small that no significant variation was found concerning the pH, the chlorinity and the amount of calcium. Then through the following 10 days, the fish were reared without food in the sea water containing $Ca^{45}$. In this experiment, half of the water in every tank was exchanged every other day for fresh sea water which maintains each concentration, temperature and amount of $Ca^{45}$ in the same conditions as before. After this rearing was concluded, the otoliths were taken out from each fish.

This kind of rearing experiment was repeated six times during the period from September, 1957 to June, 1958. Various conditions for this experiment are denoted in Table 2. Similar experiment was also carried out concerning Fish-$B_2$ (shown in Table 1).

Table 2. Conditions for Experiment-4. Each datum indicates the average value. But the item of Water temperature indicates the deviation of temperature through each experiment. And the item of Dissolved oxygen indicates the minimum value and it was 95 ~ 100% through almost all period. The amount of food taken through the preparatory rearing is denoted as follows.
FF···Taken with appetite F···Taken a little N···Taken nothing

| | | September 1957 | October | December | February 1958 | May | June |
|---|---|---|---|---|---|---|---|
| Fish length (cm.) | | 8.5 | 8.8 | 9.6 | 9.5 | 9.8 | 10.6 |
| Fish weight (g.) | | 16 | 22 | 25 | 26 | 26 | 30 |
| Feeding in preparatory rearing | | FF | FF | N | N | F | F |
| Otolith (length) × (width) (cm.) | | 0.39×0.26 | 0.41×0.28 | 0.46×0.29 | 0.46×0.30 | 0.51×0.33 | 0.53×0.33 |
| Water temperature (°C) | | 22±1 | 20±1 | 10±1 | 8±1 | 18±1 | 23±2 |
| Dissolved oxygen (% in saturating degree) | | >90 | >90 | >92 | >96 | >90 | >91 |
| A-group | Concentration of water (‰ in chlorinity) | 17 | 17 | 17 | 17 | 17 | 17 |
| | Dissolved Calcium (g./*l.*) | 0.37 | 0.36 | 0.37 | 0.36 | 0.36 | 0.36 |
| B-group | Concentration of water (‰ in chlorinity) | 14 | 14 | 14 | 14 | 14 | 14 |
| | Dissolved calcium (g./*l.*) | 0.31 | 0.31 | 0.30 | 0.30 | 0.30 | 0.29 |
| C-group | Concentration of water (‰ in chlorinity) | 10 | 10 | 10 | 10 | 10 | 10 |
| | Dissolved calcium (g./*l.*) | 0.23 | 0.22 | 0.23 | 0.22 | 0.23 | 0.22 |

*Measuring method of radioactivity.*

Each otolith removed from the fish was washed carefully not to injure its surface and was dried sufficiently in the room. Then a pair of otolith was placed in the center of a dish (2.8 cm. in diameter, 0.5 cm. in depth) which was made of stainless steel as is shown in Fig. 3-a. Radioactivity of the pair of otolith was counted $N_1$ counts/min. from one side of its surface by using a radiation counter, TOKYO SHIBAURA Electric Co. R. G. D-100-B. The distance from the mica window of G-M tube to the half height of the otolith was always kept 1.0 cm. as is shown in Fig. 3-b. Then, the pair was turned upside down and their radioactivity was counted $N_2$ counts/min. in the same way (shown in Fig. 3-c).

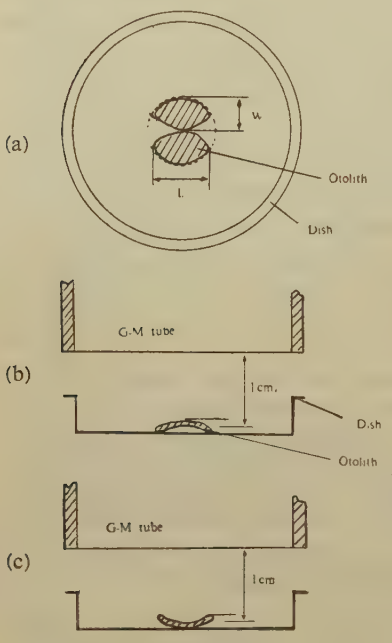


Fig. 3. (a) Top view of a pair of otolith of *Mylio* (Japanese name: KURODAI. Fish-$C_1$ & $C_2$) in the dish. (b), (c) Cross view of G-M tube, the dish and the otolith

When the radioactivity is counted from one side of the otolith, it is clear that the amount of this counts contains not only the counts of that side but also those of radioactivity penetrated from the other side. In order to make clear the amount of counts of the latter, the following experiment was carried out using many otoliths of various size. At first, the otolith was coated using dilute solution of $Ca^{45}Cl_2$, and after it was dried, radioactivity of its surface was counted T counts/min. Then, it was turned upside down and was counted t counts/min. The value of the ratio of both counts $R = t/T$ was thus found approximately to be 0.08 concerning the otolith used in the present experiment. It can be estimated therefore that total radioactivity of a pair of otolith is approximately $\frac{N_1 + N_2}{1.08}$ counts/min.

The length and the width of each otolith were measured by using microscope. L in the following researches shows the average length of the pair of otolith and W shows their average width. The pair of otolith of *Mylio* could be arranged almost uniformly in a dotted circle of radius W cm. as is shown in Fig. 3-a. Therefore, the radioactive source may be regarded approximately as a circular plane of radius W cm. Geometrical efficiency G of G-M counter was obtained by means of Cook's method[9]. The radioactivity of the standard source (producted by the Scientific Research Institute, and its disintegration, $D_\circ$ per minute) was counted n counts/min. by using the same counter. Geometrical efficiency $G_\circ$ of

the standard source was obtained by Cook's method too. If the correction for the absorption of $\beta$-rays in the mica window of G-M tube and in the air between G-M tube and the specimens, is denoted by $f_w$, the following equation will be obtained approximately.

$$D_\circ = \frac{n}{G f_w} \quad \text{(dis./min.)}$$

From this equation, the $f_w$ was calculated. The amount of total disintegration (D dis./min.) of the pair of otolith can be got as follows.

$$D = \frac{N_1 + N_2}{1.08\, G f_w} \quad \text{(dis./min.)}$$

Therefore, the amount of radioactive calcium (R $\mu$c.) contained in a whole surface of the pair of otolith can be got as follows.

$$R = \frac{D}{3.7 \times 10^4 \times 60} = \frac{4.2\,(N_1+N_1)\, D_\circ G_\circ \times 10^{-7}}{Gn} \quad (\mu c.)$$

The amount of radioactivity 3 $\mu$c./$l$. of $Ca^{45}$ which was put into the rearing water in the beginning of this experiment, will be decreased to 2.9 $\mu$c./$l$. during the following 10 days by its natural decay. When the amount of calcium contained in the water is K g./$l$., the amount of calcium Z g., taken from the sea water and deposited on the surface of the pair of the otoliths during these 10 days, is obtained from the following formula.

$$Z = \frac{KR}{2.9} = \frac{1.4\,(N_1+N_2)\, D_\circ\, G_\circ\, K \times 10^{-7}}{Gn} \quad \text{(g.)}$$

From the amount of Z of each fish, calcification rate Z' g. per $cm^2$. of the otolith surface per day was calculated from the following formula.

$$Z' = \frac{Z}{LW \times 0.70 \times 4 \times 10^{-2}} = \frac{Z}{28\,LW} \quad \text{(g./cm}^2\text{. day)}$$

In this formula, LW $\times$ 0.70 $\times$ 4 $cm^2$. was obtained through many observations of otoliths and may be regarded the approximate total surface area of the pair of otoliths in *Mylio*.

Yet, in the case of the otolith of *Lateolabrax*, the radioactive source may be regarded approximately as a circular plane of radius 1.2 $\times$ W cm. (Fig. 4).

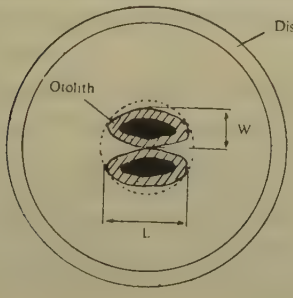


Fig. 4. A pair of otolith of *Lateolabrax* in the dish. Radius of the circle drawn by a dotted line is 1.2 W cm. and most otolith of *Lateolabrax* could be arranged almost uniformly in this circle.

Surface area of the pair of otolith was approximately $LW \times 0.74 \times 4\,cm^2$. Hence, the calcification rate Z′ was obtained as follows.

$$Z' = \frac{Z}{LW \times 0.74 \times 4 \times 10} = \frac{Z}{30LW} \quad (g./cm^2.\ day)$$

The correction for the self-absorption loss of the radioactivity was made by using Comar's data[11]. In the present study, the amount of calcium deposited upon the otolith during these 10 days was mostly less than 0.5 mg./cm². In this range, no correction was made. But, according to Comar's data, the counts were corrected 3% when the amount of calcium was 0.5 ~ 1.0 mg./cm²., and were corrected 6% when it was 1.0 ~ 1.5 mg./cm².

The central part of one side surface of various sized otolith (dark part shown in Fig. 4) was coated by using the solution of $Ca^{45}Cl_2$. But the radioactivity of this surface could not be counted from the other side. Therefore, back-scattering can be neglected concerning these otoliths used in the present experiment. And it was found that the value of R is due to the thin hem of the otolith (the part denoted by oblique line in Fig. 4), because, in the central part of the otolith, the value counted from one side did not contain the counts penetrated from the central part of the other side.

*Experiment-5*: Effect of the water temperature concerning the growth of otolith.

The following experiment was also carried out using $Ca^{45}$ as a tracer. In November, 1958, nine fish of similar size were taken out from the group of Fish-$C_2$ (shown in Table 1), and divided into three, S-, H- and M-group. Each group which has three fish was put into a rearing tank having 30 *l.* of sea water of 15°C. In 5 days, the water temperature of S-group was raised gradually to 22°C, and H-group to 20°C, while M-group was kept at 15°C constantly. The concentration of the water in every tank was kept at 17‰ in chlorinity. For the following 5 days, preparatory rearing of these fish was carried on in the tank keeping each temperature unchanged. During this period, enough food was given once a day.

When this preparatory rearing was concluded, $Ca^{45}$ ($CaCl_2$ in HCl solution) was added to every group at the rate of 3 μc. per *l.* of the sea water. Then, through the following 10 days, these fish were reared without food in the tank containing $Ca^{45}$. Through these procedures, half of the water in every tank was exchanged in the similar way mentioned before. When this rearing was concluded, the otoliths were taken out from each fish.

This kind of rearing experiments were also repeated in January, March and May, 1959. Various conditions for this experiment are shown in Table 3.

*Experiment-6*: Effect of the amount of food concerning the growth of otolith.

The following experiment was also carried out using $Ca^{45}$ as a tracer. Similar sized 10 fish were taken out from the group of Fish-$B_1$ (shown in Table 1) and were divided into two, F-and N-group. Each group which has 5 fish was put into

Table 3. Conditions for Experiment-5. FF, F and N are similar to Table 2. Activity of the fish is denoted as follows.
VS·····Moved very actively, S·····Moved sensitively,
D·····Moved dully.

| | | November 1958 | January, 1959 | March | May |
|---|---|---|---|---|---|
| Fish length (cm.) | | 9.5 | 9.5 | 9.2 | 9.7 |
| Fish weight (g.) | | 24 | 20 | 20 | 28 |
| Otolith (length) × (width) (cm.) | | 0.44×0.28 | 0.44×0.29 | 0.42×0.28 | 0.46×0.30 |
| Water temperature (°C) / Food : Activity | S-group | 22 ± 1 / FF : VS | --- | --- | 22 ± 1 / FF : VS |
| | H-group | 20 ± 1 / FF : VS | 20 ± 1 / FF : VS | 20 ± 1 / FF : VS | 20 ± 1 / FF : VS |
| | M-group | 15 ± 1 / F : S | 15 ± 1 / F : S | 15 ± 1 / F : S | 15 ± 1 / F : S |
| | L-group | --- | 10 ± 1 / N : D | 10 ± 1 / N : D | --- |
| Concentration of water (‰ in chlorinity) | | 17 | 17 | 17 | 17 |
| Dissolved calcium (g./*l.*) | | 0.36 | 0.37 | 0.37 | 0.35 |
| Dissolved oxygen (% in saturating degree) | | >96 | >96 | >99 | >93 |

a rearing tank having 30 *l.* of the sea water, and then preparatory rearing was carried on for the following 10 days giving plentiful fish meat once a day.

When the preparatory rearing was finished, $Ca^{45}$ ($CaCl_2$ in HCl solution) was added to every group at the rate of 3 $\mu$c. per *l.* of the sea water. Through the following 10 days, the fish of F-group were given food continuously, but N-group was reared without food. Every remains of food in the former case was taken out instantly. Half of the water in every tank was exchanged in the similar way as was mentioned before.

This method of rearing was repeated eight times through the period from June, 1957 to June of the next year. However, during the period from December to June, 1958, 60 *l.* of water instead of 30 *l.* was put into each tank, because the fish had grown more than 15 cm. in length. Various conditions for this experiment are shown in Table 4.

When the rearing continued for 10 days in the sea water containing $Ca^{45}$ was concluded, the otoliths were taken out from each fish. Their radioactivity was counted in the same way as was explained before. In this case, it may be inferred that $Ca^{45}$ could not be contained in the calcium compounds of given food (for instance, bone, fin etc.). Therefore, the amount of calcium Z g. taken from the sea water and deposited to the surface of the otoliths can be obtained by the above-mentioned method.

*Mylio* and *Lateolabrax* used in Experiment-2 ~ Experiment-6 were not adult but young fish reared artificially. The difference, however, could not be found

Table 4. Conditions for Experiment-6. FF, F and N denote the amount of food taken by F-group similarly to Table 2.

| | June 1957 | July | August | September | December | February 1958 | May | June |
|---|---|---|---|---|---|---|---|---|
| Fish length (cm.) | 6.5 | 7.2 | 9.1 | 10.9 | 15.3 | 16.4 | 16.3 | 16.5 |
| Fish weight (g.) | 5 | 6 | 12 | 21 | 56 | 68 | 60 | 70 |
| Amount of food of F-group | FF | FF | FF | FF | N | N | F | F |
| Otolith (length) × (width) (cm.) | 0.39× 0.21 | 0.43× 0.23 | 0.51× 0.27 | 0.62× 0.31 | 0.81× 0.41 | 0.84× 0.42 | 0.83× 0.40 | 0.89× 0.42 |
| Water temperature (°C) | 23 ± 2 | 26 ± 1 | 26 ± 1 | 22 ± 1 | 10 ± 1 | 8 ± 1 | 18 ± 1 | 23 ± 2 |
| Concentration of water (‰ in chlorinity) | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 |
| Dissolved calcium (g./l.) | 0.35 | 0.36 | 0.35 | 0.37 | 0.36 | 0.36 | 0.36 | 0.35 |
| Dissolved oxygen (% in saturating degree) | >94 | >94 | >93 | >96 | >94 | >96 | >93 | >94 |

between the zones of the adult fish and those of reared ones. Moreover, the rearing conditions were regulated as nearly as possible to natural conditions. And, it is probable therefore that no essential difference may be seen between these two kinds of fish.

## (IV) RESULTS

From the above-mentioned experiments, the following results were obtained.

(1) Laue photographs of every portion of the otolith slice were taken through the Experiment-1 and the typical examples of them are shown in Plate 1. In these Laue photographs, Fig. A, B, C, D and E are the radiographs of the portions denoted by $T_1$, $O_1$, $T_2$, $O_2$ and $T_3$ in Fig. 1 respectively. Therefore, Fig. A, C and E are radiographs of the portions in the translucent zone, and Fig. B and D are those of portions in the opaque zone. From these radiographs, the followings were obtained. The micro-crystals of $CaCO_3$ in any part of the otolith of *Argyrosomus* are in the form of aragonite and are fibrously arranged similarly to *Pseudosciaena*[7]. The size of the micro-crystal grains of $CaCO_3$ in the translucent zone is larger than that of the opaque one generally.

(2) Electron micrographs of the otolith surface were taken through the Experiment-2 and representative ones are indicated in Plate 2 and 3. Magnifications of these micrographs are all 5,000 ×. The observations obtained from these micrographs are shown in Table 5. From this table, the followings were induced.

(a) In summer and autumn, the size of crystal grains in the otolith surface is generally larger than in winter and spring.

(b) In summer and autumn, the groove between the boundaries of the grains are deeper than in winter and spring. In winter and spring, much amount of something which may be a kind of organic matter can be observed in the groove, and the surface is smooth comparatively. While it can be observed little in summer

Table 5. The surface texture of the otolith observed using the electron micrographs. Division An., M. and Po. are denoted in Fig. 2-b.

- • ……the small sized grain
- ● ……the medium sized grain
- ⊙ ……the large siged grain
- Or……much organic matter in the groove.
- × ……little organic matter in the groove.
- v ……the shallow groove between grains.
- V ……the ordinary groove between grains.
- **V** ……the deep groove between grains.

| | Division An. | Division M. | Division Po. |
|---|---|---|---|
| February | • v Or | • v Or | • v Or |
| May | • v × | • V Or | • v Or |
| July | ● V × | ● V × | ● V × |
| August | ● **V** × | ● **V** × | ● **V** × |
| September | ⊙ **V** × | ⊙ **V** × | ⊙ **V** × |
| December | ● V × | • V × | ⊙ V × |

and autumn.

(c) No significant difference is found between the divisions, An., M. and Po.

(3) The micro-autoradiographs of the otolith slice were taken through the Experiment-3 and typical one of them is shown in Fig. A of Plate 4. It is clear that the darkness of these zones depends on the amount of $Ca^{45}$ deposited to the otolith. Fig. B of Plate 4 is a photomicrograph of the same slice, and it can be seen that the whole area of this slice is translucent except its central area. Fig. 5

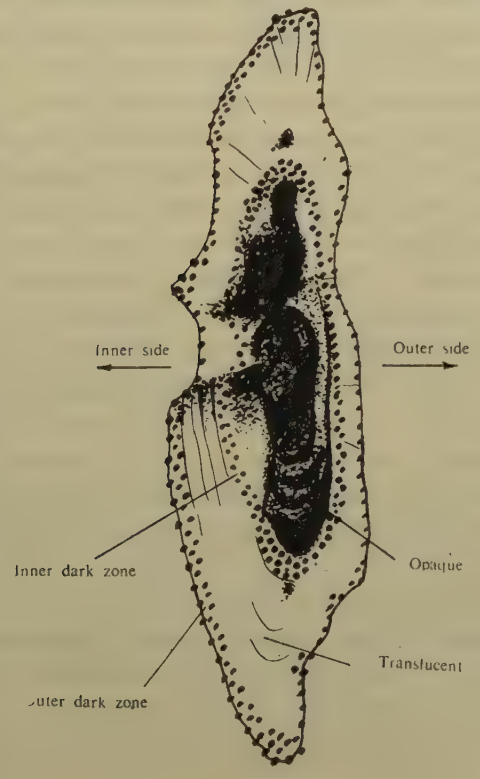


Fig. 5. The otolith slice of *Lateolabrax* (Fish-$B_3$) and its autoradiograph. Dark central part written with small dotts is a opaque part of the slice and the other part is translucent. Two zones written with large dotts are the dark zones of the autoradiograph.

is a figure traced by putting Fig. A upon Fig. B. As is shown in this figure, the shape of the outer hem of the outer dark zone is nearly the same with that of the edge of the slice, and that of inner hem of the inner dark zone is nearly the same with that of the outer edge of the opaque zone. Between those two dark zones, a light zone whose breadth is more than two times of these dark zones can be seen. Accordingly, it can be inferred that the inner and outer dark zones were produced by $Ca^{45}$ taken from the rearing water and deposited on the surface of the otolith in June and September, and the opaque zone in the central part of this slice was already formed before the experiment in June was begun.

Moreover, those two zones which can be seen in the upper, lower and left part (inner side of the actual otolith as is shown in Fig. 2-a) are darker and wider than those in the right part (the outer side of the actual otolith). And concerning the distance between these two zones it was observed that the largest is the upper and lower, the smallest is the right and the medium is the left part. Similar results were found also in the radiographs of the otolith in the fish reared twice in the rearing tank containing $Ca^{45}$ in September and November. Accordingly, it may be concluded that the otolith grows mainly by depositing upon its surface calcium contained in the sea water, and that, concerning the rapidity of its growth, the largest is the two extreme ends of the otolith, the smallest is its outer side, and the medium is its inner side.

In Fig. 5, it is seen that the outer dark zone of radiograph protrudes more or less beyond the outer edge of the slice. Such a little disagreement may be due to the fog of the radiograph. And the same fog can be imagined to exist on all hem of these two zones. Therefore, though the hem line is not sharp but rather dim, it is imagined that the two zones exist in a definite and clear shape. This fact shows that otolith grows piling up the layer gradually for outward direction. The boundary between the inner hem of the inner zone and the outer edge of the opaque zone is to show the turning point in its growth from the piling of the opaque layer to that of the translucent layer, but it is hard to examine its fine structure minutely owing to the inaccuracy of this radiograph. It was confirmed originally and systematically for the first time through these experiments, however, that the opaque zone was already formed before June every year, and the translucent zone was begun to be formed from June and was continued to September of the same year.

(4) Concerning the concentration of the sea water and the calcification rate $Z'$ of the otolith, the results shown in Fig. 6-a were obtained through Experiment-4. Water temperature in this experiment was shown in Fig. 6-b. From those figures, the followings were found.

(a) In any season, no significant difference of the calcification rate was discovered between A-, B- and C-group. In other words, the calcification rate of the otolith of *Mylio* and *Lateolabrax* is independent of the concentration of the sea water surrounding the fish.

(b) From June to October, the calcification rate of the otolith is larger than

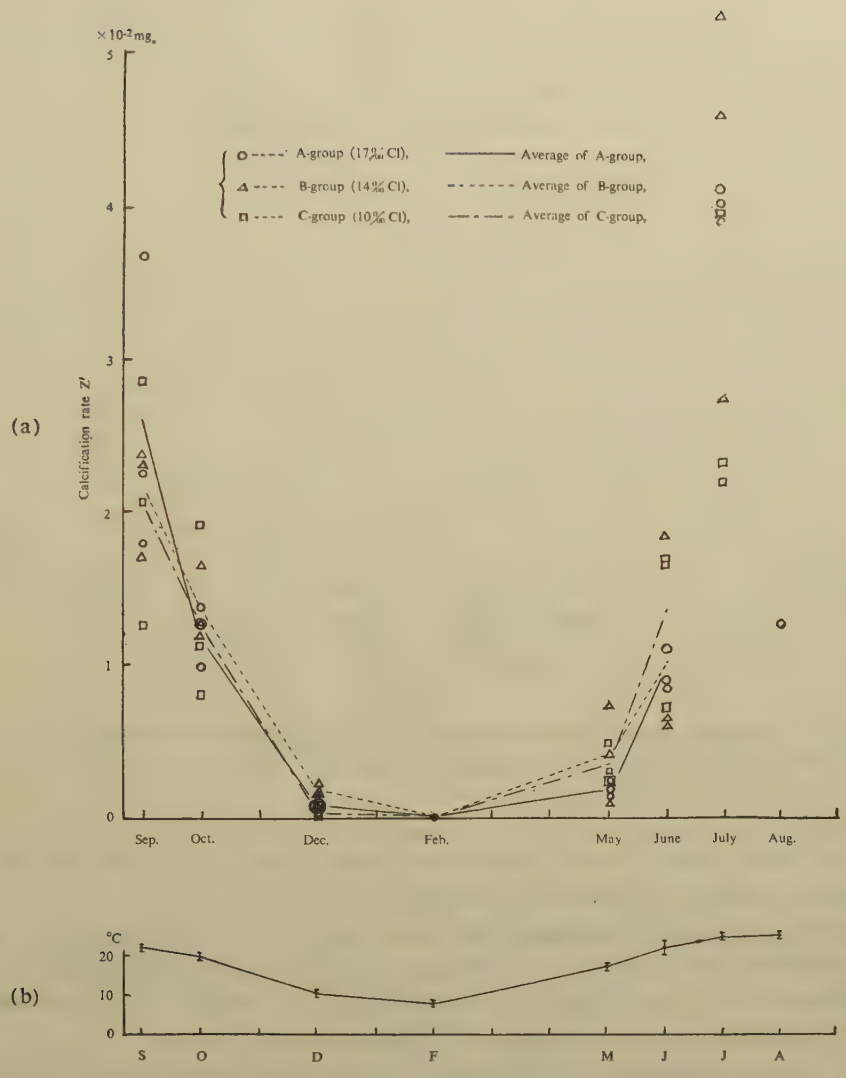


Fig. 6. (a) Seasonal changes of calcification rate Z′ mg./cm². day of the otolith of Fish reared in various concentrations of the sea water (Exp.-4). Values in July were obtained by using *Lateolabrax* (Fish-$B_2$), and the others were *Mylio* (Fish-$C_1$). (b) Temperature of the rearing water in each experiment.

in other months. Especially, in February and March, it becomes very small.

(c) In any case of concentration of the rearing sea water, calcification of the otolith is seen scarcely when the water temperature is below 10°C, a little when it is about 15°C and conspicuously when it is above 20°C.

(5) Concerning the water temperature and the calcification rate Z′ of the otolith, the results shown in Fig. 7 were obtained through Experiment-5. Values of Z′ in September, October, December and February were reproduced from the value of A-group (17‰) in Fig. 6-a. The activities of the fish in the tank were

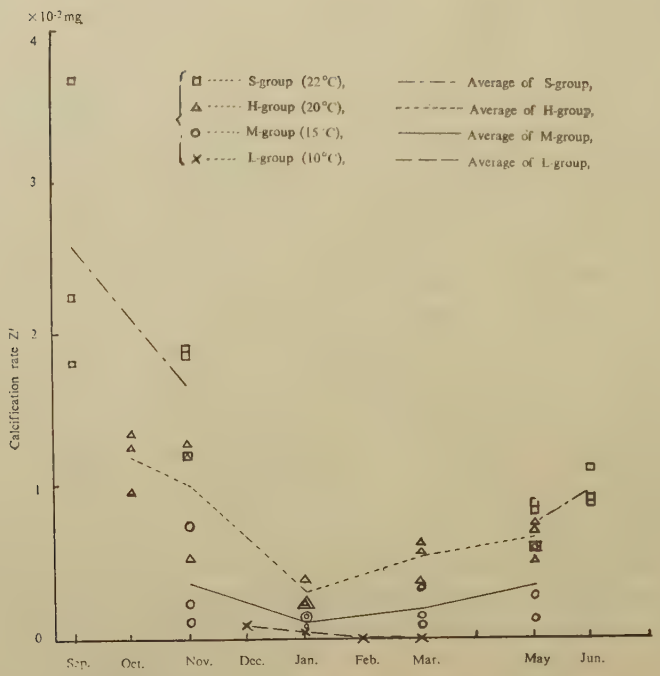


Fig. 7. Seasonal changes of calcification rate Z′ mg./cm². day of the otolith of *Mylio* (Fish-$C_2$) reared in various water temperature (Exp.-5).

shown in Table 3 (item: Water temperature/Food: Activity). From both this figure and the table, the followings were found.

(a) The calcification rate of the otolith of *Mylio* is to be found, leastly in the water of 10°C, a little at 15°C and high at above 20°C. But in each case of the temperature, it is also affected by the season, and it is natural that the smallest is January and February, and the largest is September and October.

(b) The calcification rate of the otolith is small when the behavior of fish is dull, but is large when its activity is sensitive.

(6) Concerning the amount of food and the calcification rate Z′ of the otolith, the results shown in Fig. 8-a were obtained through Experiment-6. From this figure the followings were gained.

(a) The calcification rate of the otoliths of *Lateolabrax* in summer and autumn becomes low in winter and spring.

(b) The calcification rate of the otolith in the fish which took food plentifully is higher than those given food scantly.

(c) The calcification rate is low when the water temperature is below 10°C, and is very high when it is above 20°C.

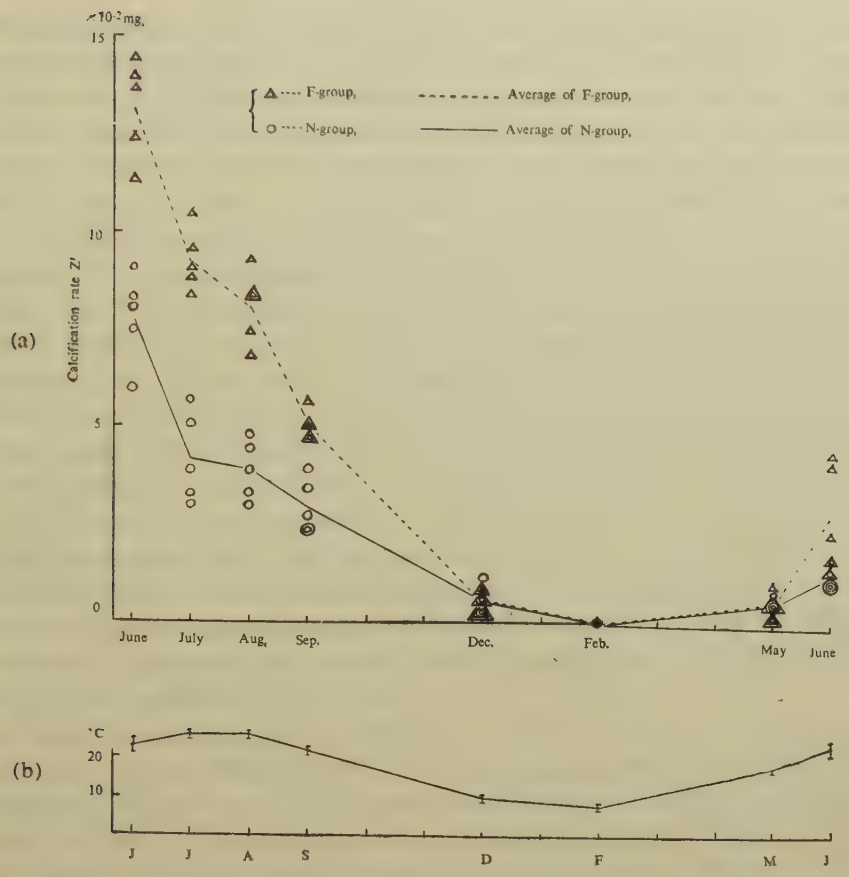


Fig. 8. (a)Seasonal changes of calcification rate Z′ of the otolith of *Lateolabrax* (Fish-$B_1$) reared with various amount of food (Exp.-6). The fish of F-group were given food sufficiently, while those of N-group were given nothing for 10 days. (b) Temperature of the rearing water in each experiment.

## (V) DISCUSSIONS

### (1) The Crystal Texture and the Growth of the Otolith.

Previously, the crystal texture and the arrangement of $CaCO_3$ which is the main constituent of the otolith were examined by means of X-rays and others. And it has already been elucidated by the writer[7] that the opaque zone which forms a part of the "annual ring of the otolith" together with the translucent zone, is formed in winter and spring, the latter is formed in summer and autumn, and a kind of organic matter (Protein[10]) is contained more abundantly in the opaque zone than in the translucent one.

In the present study, discriminating between translucent zone and opaque one, size and texture of micro-crystal grains were examined by using micro-beam X-rays (0.09 mm. in diameter) emitted from a micro-focus and by using electron microscope. As the result, it was found that micro-crystal grains in any zone are

crystalline $CaCO_3$ having the form of aragonite and are fibrously arranged as is mentioned in the first part of the results. It was also found firstly that, in the translucent zone, the size of micro-crystal grains is larger than that in the opaque zone; secondly, that only small amount of protein sticks to the groove between each grains of the translucent zone and therefore groove is deep, while, on the other hand, in the opaque zone, much protein fills the groove and covers the surface of the grain and therefore the groove is shallow and the surface of the grain becomes rather smoother than that of the translucent zone. Moreover, through the measurement of calcification rate of the otolith surface, it was found that the calcification rate becomes high in summer and autumn through which seasons the translucent zone is thus formed, while it becomes low in winter and spring through which the opaque zone is formed consequently. The present writer could only infer in the previous research[7] that the translucent zone may due to the vigorous calcium deposition and the opaque zone may due to its slow deposition, but through the present experiments it was first ascertained to be a definite true. At the same time, it was examined by using X-rays that crystalline $CaCO_3$ in the form of aragonite is formed by combining calcium with organic matter surrounding the otolith. The autoradiograph obtained originally in this experiment shows that most part of the calcium is taken from the sea water and is deposited on the whole surface of the otolith, and that the amount of its deposition in this case differs by the place remarkably. In other words, calcium is deposited, abundantly on the two extreme ends, leastly on the outer side, and mediumly on the inner side. Therefore it may be concluded that the growth of the otolith differs locally, that is to say, the rapid growth can be found towards both ends, slow growth towards the outer side, and the medium growth towards the inner side. Moreover, it was elucidated that the zone mark formed in the otolith once is preserved ever after as it is, and the form of the zone indicates what the shape of the otolith was when the zone was formed.

Lastly, the followings are inferred concerning the formation of the translucent and opaque zone in the otolith. The number of the micro-crystal grains of $CaCO_3$ increases a little in winter and spring and their size is small because the amount of protein surrounding the otolith is much in comparison with that of calcium deposited on the surface in these seasons. Therefore, the groove between these crystal grains is filled with much protein, and the visible rays can hardly penetrate. On the other hand, in summer and autumn, the number of the crystal grains increases rapidly and their size is large because the otolith is surrounded with a less amount of protein in comparison with calcium. In this case, the groove between crystal grains are filled with less protein, and therefore, the zone formed in these seasons becomes translucent to visible rays. It is thus clear that the otolith grows by piling up the translucent layer and opaque layer alternately.

In winter, the calcification rate of *Mylio* is exceedingly low than that of *Lateolabrax*. It can be inferred therefore that the formation of the micro-crystal grains in this season is almost stagnated, and the surface of the otolith remains

almost unchanged. It may be due to this reason perhaps that in the margin of the otolith of *Mylio* caught in winter the opaque zone could not be found in the previous research[8] by the present writer. It must be avoided to estimate the season for the formation of these zones only through the zone in the margin of the otolith.

(2) Growth of the Otolith and the Living Condition of the Fish.

As is mentioned above, the growth of the otolith is due to the formation of crystal grains of $CaCO_3$ caused by the deposited calcium upon the surface of the otolith. It was once said that this calcium might be taken from the food[3]. But, it was for the first time found through the present experiments that a large amount of calcium contained in the sea water is also deposited clearly upon the surface of the otolith. It was elucidated moreover that the amount of calcium taken from the sea water and deposited upon the otolith is quite independent of the concentration of the sea water, and that it becomes small below 15°C of water temperature and becomes remarkably large above 20°C. The deposition of calcium, that is to say, the formation of crystal grain of $CaCO_3$, was found to be affected greatly by season even when the rearing water is kept its temperature unchanged or when its concentration unchanged. In any case, it may be concluded that calcium contained in the sea water is deposited abundantly in summer and autumn and a little in winter and spring.

One of the conspicuous difference between the fish in the period from summer to autumn and the one from winter to spring is that of their activity. In winter and spring, the behavior of the fish is dull, but it becomes sensitive and active in summer and autumn. It can be said therefore that the activity of the fish is closely connected with the amount of calcium taken from the sea water and deposited upon the surface of the otolith. On the other hand, in the present experiment, no significant difference in their activity was found between the fish which took food plentifully and that took insufficiently. Nevertheless, the amount of calcium deposited on the otolith of the former was exceedingly larger than that of the latter. Accordingly, it is considered that the speed for the increase of crystal grains of $CaCO_3$ in the otolith, in other words, the growing velocity of the otolith, is not due to their external activity but to their physiological condition. Here it may be concluded that when the environment of the fish is favorable to their living and their physiological condition is good, the otolith grows rapidly by taking up a large amount of calcium from the sea water. On the contrary, when their environment is unfavorable and their physiological condition is not good, the otolith grows slowly. Both physiological conditions, good and not good, can be realized either in summer and autumn or in winter and spring; moreover, it is clear that calcium is taken and deposited to the otolith surface not only from the sea water but also from their food. Therefore, when the calcium contained in the food was measured together with the calcium in the sea water, more conspicuous effects might be expected to be observed.

## (VI) SUMMARY

In order to elucidate how the crystal texture of the fish otolith and its growth are influenced by water temperature, its concentration, the amount of food and the season, the present writer chose marine teleosts, *Lateolabrax* and *Mylio*. They were reared through all seasons in tanks and the temperature of the sea water, its concentration and the amount of food were variously changed and experimented. For the observation of the crystal texture of the otolith surface, the electron microscope was used and details concerning the formation of the otolith zone were studied through the autoradiographs. Radioactive isotope $Ca^{45}$ was used as a tracer in order to measure the amount of calcium deposited upon the otolith surface, and, concerning the crystal texture of the annual rings, *Argyrosomus* was used instead of *Lateolabrax* and *Mylio* for convenience' sake, and their Laue photographs were taken by the micro-beam X-rays. As the results, the followings were obtained.

(1) Calcium dissolved in the sea water is deposited on the surface of the otolith, and micro-crystal grains of $CaCO_3$ which are the main constituent of the otolith are thus formed. It goes without saying that the growth of the otolith is mainly due to the formation of micro-crystal grains of $CaCO_3$.

(2) In summer and autumn, in which the fish grows rapidly, a large amount of calcium is deposited on the otolith surface, and the micro-crystal grains of $CaCO_3$ are formed abundantly. A very little protein fills the groove between crystal grains. In this way, the wide translucent zone which contains a small amount of protein is formed.

(3) In winter and spring, in which the fish grows slowly, a small amount of calcium is deposited and the micro-crystal grains are formed very slowly. Much protein fills the groove between crystal grains. This is the way in which the opaque zone containing much protein is formed.

(4) Concerning *Lateolabrax* and *Mylio*, the concentration of the sea water or the amount of the dissolved calcium in this water cannot have influence upon the formation of micro-crystal grain of $CaCO_3$. In other words, the growing velocity of the otolith is always unchanged. But, on the other hand, the formation of the micro-crystal grains is influenced much by the water temperature and the amount of food taken by the fish. The micro-crystal grains of $CaCO_3$ are formed abundantly and the otolith grows rapidly when the environment of the fish is favorable and its physiological condition is good independently of the concentration of the water. On the contrary, when their environment is unfavorable, the micro-crystal grains are formed only a little and the otolith grows slowly. The good environment for the fish in this case means that they are living in the warm water and taking plentiful food, and the contrary means that they are living in the cold water and taking only insufficient food, and both environments can be realized either in summer and autumn or in winter and spring.

(5) As is mentioned above, the formation of the zone in the otolith is closely

connected with the history of the fish and the zone mark once formed in the otolith is preserved ever after as it is. Accordingly it can be used as an effective key to investigate the history of the individual fish.

## ACKNOWLEDGEMENT

The writer wishes to express his sincere thanks to Professor Takeo Fujiwara, under whose kind guidance and encouragement this study was carried out. The writer is also grateful to Professor Mutsuaki Shinagawa for his kind guidance concerning the measuring of radioactivity. The present study was supported by the Scientific Research Expenditure of the Ministry of Education.

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## EXPLANATION OF PLATES

Plate 1 X-ray Laue photographs of the otolith thin slice of *Argyrosomus*. Fig. A, C and E are radiographs of the portions denoted respectively by $T_1$, $T_2$ and $T_3$ (translucent zone) in the Fig. 1. Fig. B and D are the ones denoted by $O_1$ and $O_2$ (opaque zone) similarly.

Plate 2 and 3 Electron micrographs of the inner side surface of the otolith. The fish used here are *Lateolabrax* (Fish-$B_2$). The name of the month when the otolith was taken out, and the division of the otolith of which the micrographs were taken are shown in the following table (5000×).

| Month | Plate | Division An. | Division M. | Division Po. |
|---|---|---|---|---|
| February | Plate 2 | Fig. A. | Fig. B. | Fig. C. |
| May | Plate 2 | Fig. D. | Fig. E. | Fig. F. |
| July | Plate 2 | Fig. G. | Fig. H. | Fig. I. |
| August | Plate 3 | Fig. J. | Fig. K. | Fig. L. |
| September | Plate 3 | Fig. M. | Fig. N. | Fig. O. |
| December | Plate 3. | Fig. P. | Fig. Q. | Fig. R. |

Plate 4 Fig. A Micro-autoradiograph of the otolith slice of *Lateolabrax* (Fish-$B_3$). The age of this fish was O and was reared in the water containing radioisotope $Ca^{45}$ twice for 10 days in June and September respectively. (22×).
Fig. B Photo-micrograph of the otolith slice used in Fig. A. It was taken through penetrated visible rays. (22×).

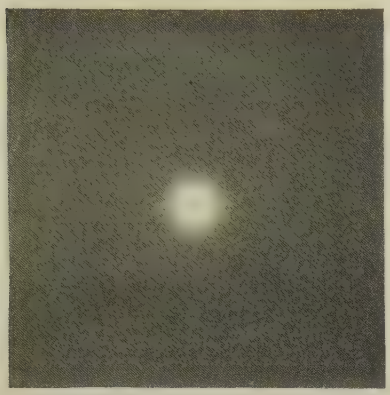


Fig. A

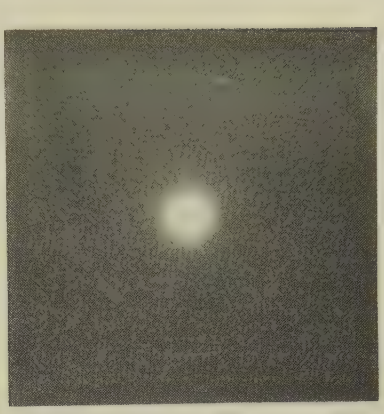


Fig. B

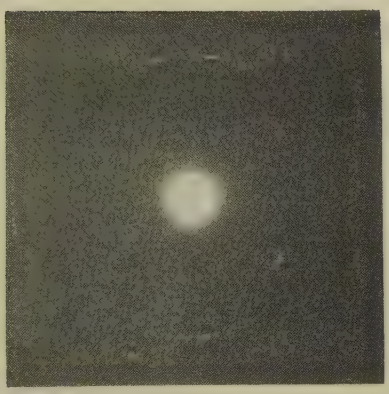


Fig. C

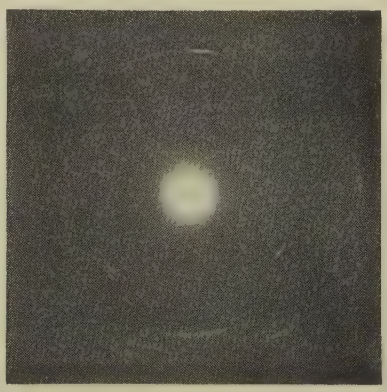


Fig. D

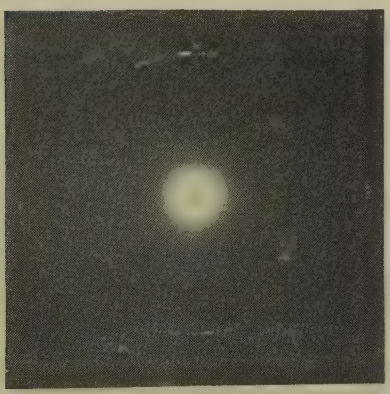


Fig. E

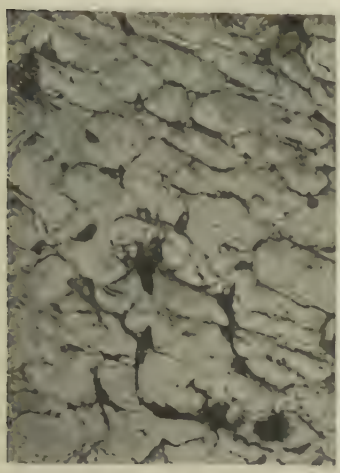


Fig. A

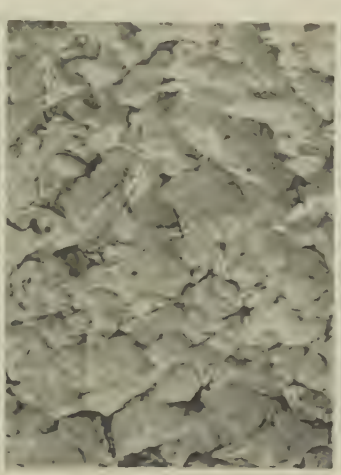


Fig. B

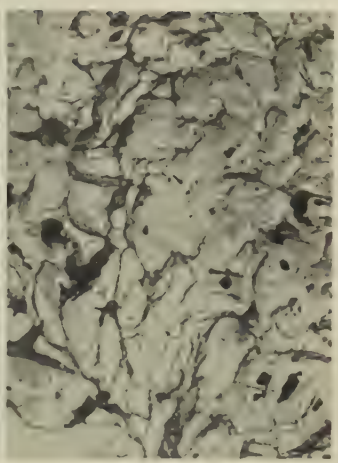


Fig. C

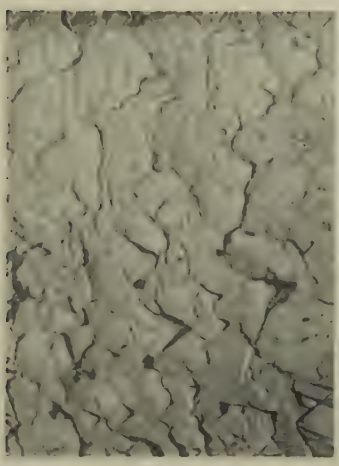


Fig. D

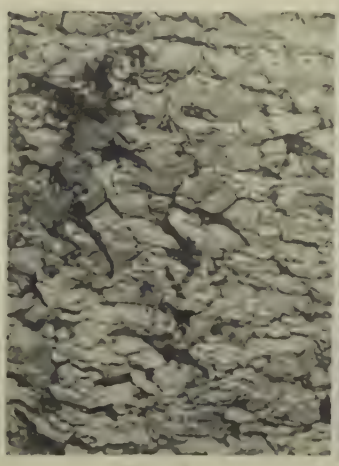


Fig. E

Fig. F

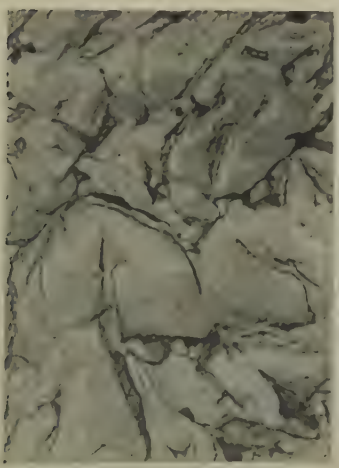


Fig. G

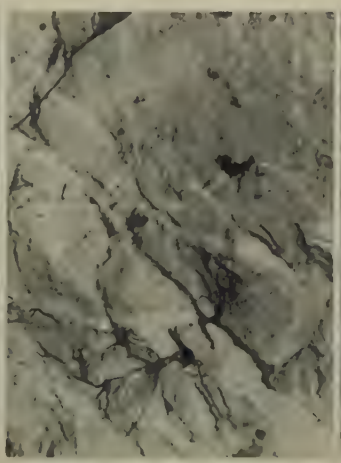


Fig. H

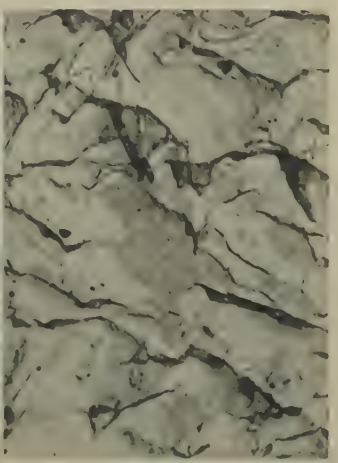


Fig. I

IRIE: The Growth of the Fish Otolith

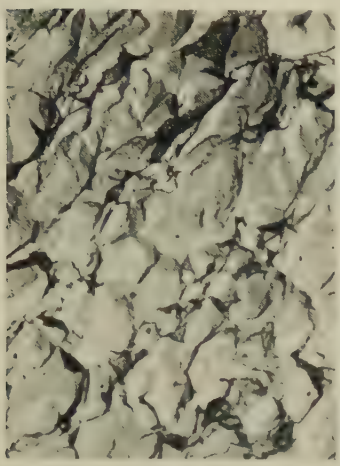


Fig. J

Fig. K

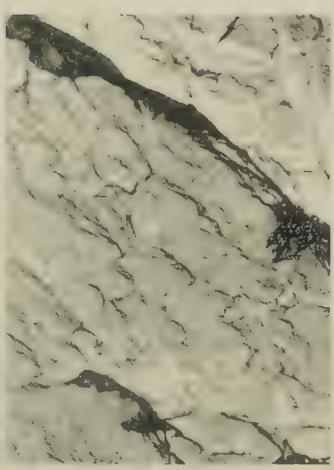


Fig. L

Fig. M

Fig. N

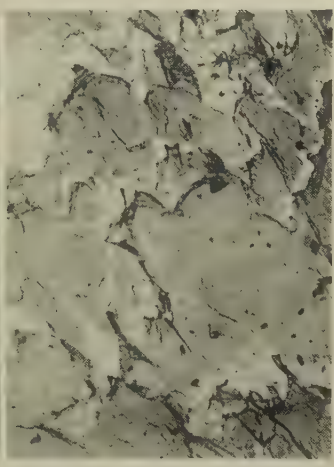


Fig. O

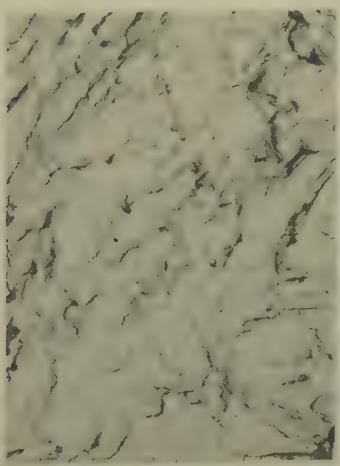


Fig. P

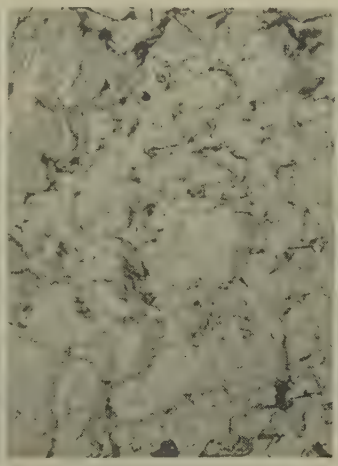


Fig. Q

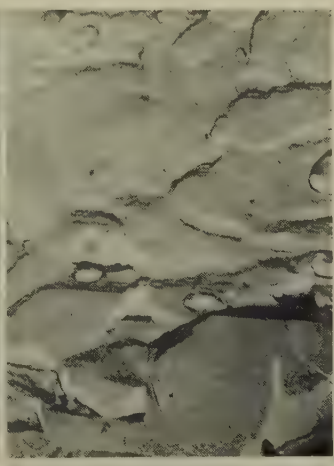


Fig. R

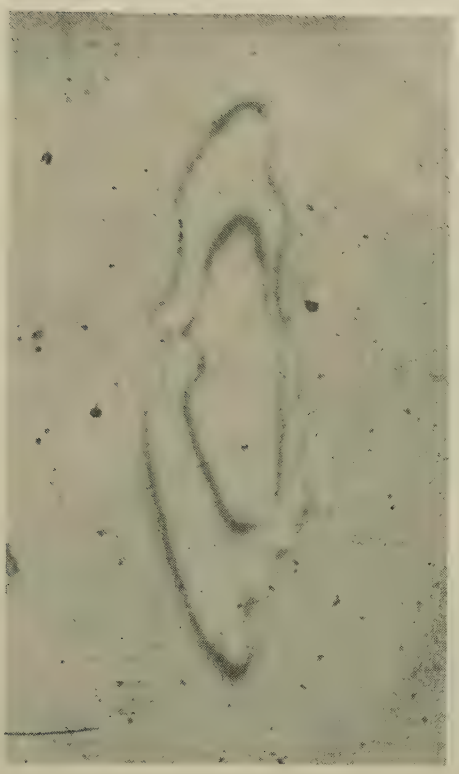


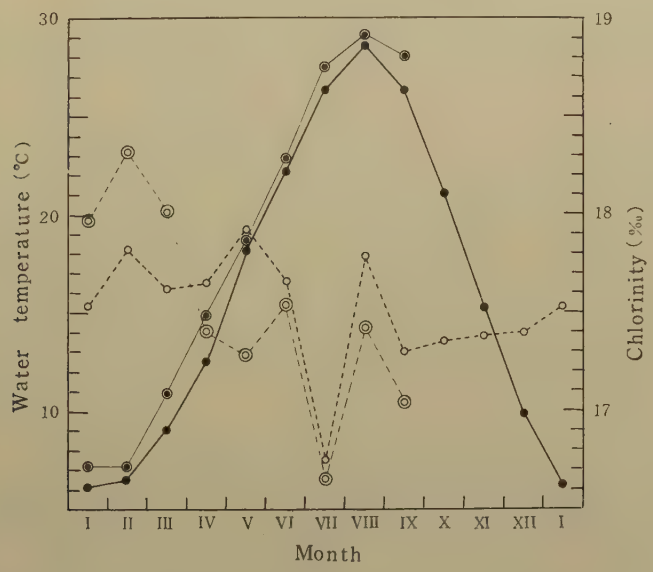
Fig. A

Fig. B

## Vol. 3 No. 2　正　誤　表　Errata

| Page | Line | Error | Correct |
|---|---|---|---|
| 247 | 5 from bottom | この水域の海域 | この水域の海況 |
| 250 | Table 3, 3～8 | 2 2, 2 08, 2 11,… 2 18, 2 16 | 2.2m, 2.08m, 2.11m,… 2.18, 2.16 |
| 250 | Table 3, 9 | 0 37, 0 41 | 0.37, 0.41 |
| 252 | Text-fig. 3, body | (Should be replaced with the figure printed below.) | |
| 252 | Text-fig. 3, title | meas | means |
| 265 | 20 | 釜屋入江前 (St. 6) | 釜屋入江前 (St. 5) |
| 270 | 2 from bottom | 西南部の西南部の | 西南部の |
| 279 | Text-fig. 14, title | Herbor | Harbor |
| 280 | Table 7, footnote line 3 | Forestry[25]) | Forestry[28]) |
| 293 | Table D-1, Cl, last line | 17. 47 | 17. 42 |
| 296 | Table F, St. K4, $\theta_W$, 8m | 24. 8 | 22. 8 |
| 306 | 10 | *articus* | *arcticus* |
| 306 | 5 from bottom | *vulgare* | *vulgaris* |
| 307 | 5 | *vulgare* | *vulgaris* |
| 308 | 13 | it | its |
| 315 | 15 | *Megele-* | *Megale-* |

(Body for Text-fig. 3, p. 252)



# Breaking Strength of a Knot of Fishing Net as Affected by the Angle between Legs of Mesh.

Shunpei KAKUDA

*Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Pls. 1–2; Text-figs. 1–7; Tables 1–6)

## INTRODUCTION

It is generally known that a netting is more breakable at knots than at any other part when a overload is applied to it.

The breaking strength of knotted netting cords has been studied by TAUTI,[1] HONDA[2)3)], SHIMOZAKI[4] and others. TAUTI analyzed theoretically the tendency that a netting cord loses part of its breaking strength when knotted, and ascribed its cause to the fact that the cord is bent at the knot. He also showed experimentally that breaking strength of a cotton cord becomes smaller when it is made into a loop of a certain diameter by itself or tied into a trawler or flat knot with another piece of cord than when it is straight. Breaking strength of netting cords of various materials tied into a trawler or flat knot was investigated by HONDA,[2)3)] SHIMOZAKI [4] and others. In their experiments the four legs of a knot were oriented as in a mesh that is fully stretched either longitudinally* or transversely*, and static loads were applied to them in the direction of the legs. KONDO[5] investigated the breaking strength of non-shortened nets.

When a properly designed fishing net is used in water, its meshes are spread open to the extent that has been planned in designing. Hence, it seems desirable that breaking strength of a netting should be investigated with its mesh spread open to various extents. Such a study, however, has not been published.

In the present study the breaking strength of a netting cord tied into a trawler or flat knot was investigated by varying the angle between the neighboring legs of the knot in order to clarify how the breaking strength of a netting is affected by the spreading of the mesh.

## MATERIAL AND METHODS

The specifications of the netting cords used in the present study are indicated in Table 1. While cotton is a vegetable fiber, cremona is a synthetic fiber (poly-

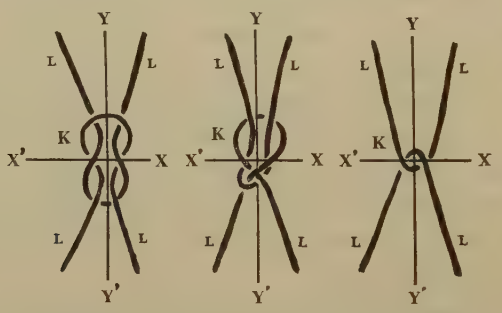
* Respectively corresponds to the case of $\alpha=0°$ and $\alpha=180°$ in Table 2 of this paper.

vinyl alcohol fiber). Cotton 1 and 2, as well as Cremona 1 and 2, are made with the same yarn and fiber and differ from one another only in the number of twists per unit length. The netting cord is cut into pieces about 35 cm long. Two such pieces of the same cord are tied into a flat or trawler knot in the middle, and are called a test piece. A test piece therefore consists of a knot and its four legs. In the case of "Plain link", a test piece consists also of two 35 cm pieces of the same cord, but one piece is simply hung over the other in the middle (Fig. 1).

Table 1. Specifications of netting cords used in the experiment.

| Cord | Material | Count | Ply/No. of yarn | No. of twist per* 10 cm. of length | | Diameter* (mm.) | Weight* per 100cm. of length (mg.) | Breaking* strength (kg.) | Breaking* elongation (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Upper twist | Lower twist | | | | |
| Cotton 1 | Cotton | No. 20 | 3/3 | 32±0.9 | 95±5.3 | 0.78±0.02 | 329±11.4 | 5.38±0.409 | 32.2±1.56 |
| Cotton 2 | Cotton | No. 20 | 3/3 | 19±0.7 | 51±3.4 | 0.85±0.02 | 300± 7.8 | 5.66±0.182 | 24.6±0.98 |
| Cremona 1 | Cremona | No. 20 | 3/3 | 21±0.8 | 57±1.7 | 0.82±0.02 | 301± 6.4 | 7.61±0.402 | 27.8±0.62 |
| Cremona 2 | Cremona | No. 20 | 3/3 | 17±0.7 | 49±2.8 | 0.85±0.02 | 295± 3.0 | 8.16±0.327 | 22.6±0.44 |

* The mean and standard deviation based on the measurements of 15 test pieces are shown.




A. Flat knot B. Trawler knot C. Plain link

Fig. 1. Sketch of the test pieces. K, knot or crossing poin; L, leg; XX′ transversal axis; YY′, longitudinal axis.

The testing machine used is a Schopper-type tensile strength tester, the upper and the lower chuck of which are replaced respectively with a horizontal bar having two horizontally movable chucks on it. The pair of testing bars are set parallel to one another on a vertical plain. A test piece is fixed to the testing bars in the manner indicated in Fig. 2.

The chucks **A**, **B**, **C** and **D** are set at an equal distance $d/2$ from the longitudinal axis of the shaft, and the length of each leg (from the chuck to the knot or crossing point K) is made to 10 cm*. (*i.e.*, $l=10$ cm.). Consequently the four legs are arranged symmetrically with regards to both the longitudinal and transverse axes passing K ($YY'$ and $XX'$). Legs are fastened to chucks in such a way that no

* See the foot notes * and ** of Table 2 for the exceptional cases.

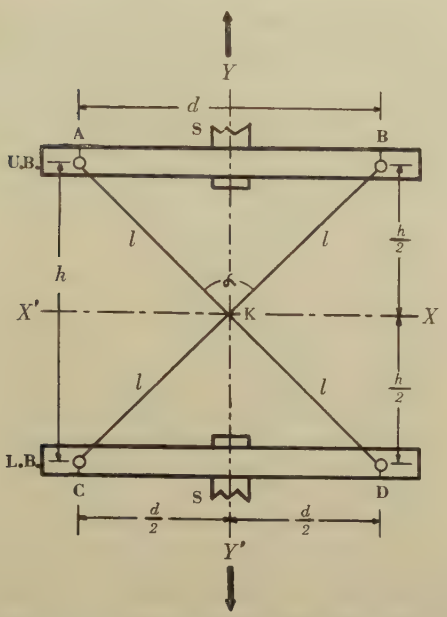



Fig. 2. A test piece fixed to the testing bars.

**A**, **B**, **C**, **D**, chucks; *d*, distance between the neighboring chucks; *h*, distance between upper and lower bars; K, knot or crossing point; **L. B.**, lower bar; *l*, length of a leg; **S**, shaft; **U. B.**, upper bar; $\alpha$, angle between neighboring legs; *XX'*, transversal axis; *YY'*, longitudinal axis.

twisting occurs at the knot or crossing point. A pair of legs represented by a single cord are fastened to the chucks of the same bar. The angle between a pair of legs represented by the single cord, $\alpha$, is varied so as to reproduce a fully spread mesh of the nettings shortened to approximately 10–80%, by varying the distance *d*.

When a test piece has been fixed to the testing bars, static loads are applied to it by moving the lower bar forcibly downwards (i.e., in the direction of *Y'*) at the constant rate of 30 cm per minute, until the test piece is broken. As the load is increased, legs of the test piece are elongated by tension, while the distance *d* remains unchanged; as a result, the angle between a pair of legs fastened to the same bar decreases and finally reached $\alpha'$ (the "critical angle") when the test piece is broken. The amount of the static load, the length of the legs and the distance between the upper and lower bars at the moment the test piece is broken are respectively denoted by *Fc* (the "critical static load"), $l'$ and $h+\Delta h$ (Fig. 3).

Test pieces are broken almost always at the knot or crossing point. In such cases the values of *Fc* and $\Delta h$ are recorded. The experiments were carried out at room temperature of $20\pm3$°C using wet test pieces which had been immersed in fresh water for 24 hours at temperatures of $20\pm2$°C.

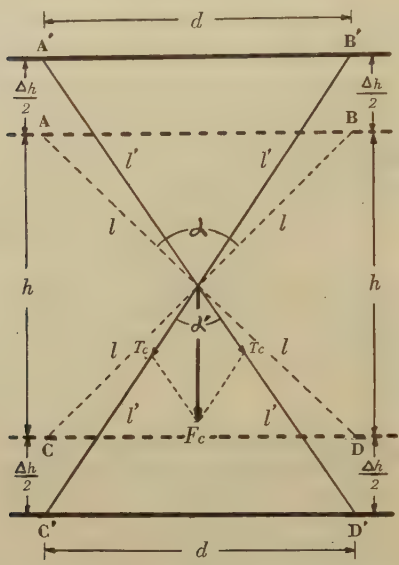



Fig. 3. Schematic representation of a test piece before stretching and at the time of its break. The broken line represents the bars and the test piece before stretching. The solid line represents those at the time of the break. *Fc*, critical static load; *Tc*, tension of a leg by *Fc*; $\alpha'$, critical angle.

## RESULTS AND DISCUSSION

The results obtained by the method described above are shown in Table 2.

Let the tension caused on a leg of the test piece by the critical load *Fc* be denoted by *Tc*. Then

$$Tc = \frac{Fc}{2} \cdot \sec\frac{\alpha'}{2} \quad . \tag{1}$$

Since $\cos\frac{\alpha'}{2} = \frac{h/2+\Delta h/2}{l'}$ and $l' = \frac{\sqrt{(h+\Delta h)^2+d^2}}{2}$, we can write (1)

$$Tc = \frac{Fc\sqrt{(h+\Delta h)^2+d^2}}{2(h+\Delta h)} \quad . \tag{2}$$

As values of *Fc*, *d* and $h+\Delta h$ are known, *Tc* can be calculated from this formula.

The breaking strength of a test piece, *BS*, in case of $\alpha'$ is put to $2Tc$.

$$BS = 2Tc$$

1) *Breaking strength in the "plain link".*

In the test piece of the "plain link" (Fig. 1C), the netting cord is broken at the crossing point in the majority of the cases, when the static load is increased gradually. The breaking strengths of the Cotton 2 and Cremona 2 cords for such cases are

calculated from the data of Table 2A by means of formula (2) and plotted against the angle $\alpha$ in Fig. 4.

TAUTI[1] has shown theoretically and experimentally that when a straight cord is bent along a circle, its breaking strength decreases and the breakage tends to occur at the bending, and also that the decrease in the breaking strength is the function of the radius of curvature of the cennter line of the bent cord.

Table 2. Means and standard deviations of $Fc$ and $\Delta h$ based on 20 test pieces.

A. Plain link

| $\alpha$ | | | 23° | 35° | 47° | 60° | 74° | 89° | 106° | 128° |
|---|---|---|---|---|---|---|---|---|---|---|
| Shortening (%) | | | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |
| Cotton 2 | $Fc$ (kg.) | Mean | 9.40 | 9.18 | 9.16 | 9.03 | 8.84 | 8.78 | 8.53 | 7.96 |
| | | s | 0.522 | 0.531 | 0.386 | 0.358 | 0.615 | 0.671 | 0.503 | 0.383 |
| | $\Delta h$ (cm.) | Mean | 5.1 | 5.2 | 5.4 | 5.7 | 6.1 | 6.7 | 7.7 | 9.2 |
| | | s | 0.23 | 0.19 | 0.13 | 0.20 | 0.22 | 0.23 | 0.20 | 0.16 |
| Cremona 2 | $Fc$ (kg.) | Mean | 8.55 | 8.87 | 8.57 | 8.88 | 8.41 | 8.45 | 8.32 | 7.76 |
| | | s | 0.828 | 0.748 | 0.861 | 0.871 | 0.578 | 0.718 | 0.595 | 0.564 |
| | $\Delta h$ (cm.) | Mean | 4.2 | 4.5 | 4.7 | 5.1 | 5.4 | 6.2 | 7.4 | 9.1 |
| | | s | 0.34 | 0.14 | 0.21 | 0.62 | 0.18 | 0.21 | 0.22 | 0.13 |

B. Trawler knot.

| $\alpha$ | | | 0°* | 23° | 35° | 47° | 60° | 74° | 89° | 106° | 128° | 180°** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shortening (%) | | | 100 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 | 0 |
| Cotton 1 | $Fc$ (kg.) | Mean | 8.64 | 8.69 | 8.44 | 8.38 | 8.23 | 7.96 | 7.44 | 7.22 | 6.71 | 9.45 |
| | | s | 0.521 | 0.819 | 0.579 | 0.674 | 0.640 | 0.445 | 0.443 | 0.604 | 0.525 | 0.833 |
| | $\Delta h$ (cm.) | Mean | 15.7 | 7.0 | 7.1 | 7.4 | 7.8 | 8.3 | 8.7 | 10.1 | 11.7 | 16.1 |
| | | s | 0.33 | 0.22 | 0.21 | 0.21 | 0.22 | 0.18 | 0.20 | 0.21 | 0.19 | 0.43 |
| Cotton 2 | $Fc$ (kg.) | Mean | 9.72 | 9.56 | 9.44 | 9.33 | 9.08 | 8.63 | 8.20 | 7.71 | 7.14 | 9.95 |
| | | s | 0.690 | 0.660 | 0.652 | 0.714 | 0.434 | 0.439 | 0.493 | 0.567 | 0.379 | 0.750 |
| | $\Delta h$ (cm.) | Mean | 12.3 | 5.4 | 5.5 | 6.0 | 6.3 | 6.5 | 7.2 | 8.0 | 9.7 | 12.2 |
| | | s | 0.30 | 0.25 | 0.16 | 0.17 | 0.27 | 0.16 | 0.28 | 0.26 | 0.17 | 0.37 |
| Cremona 1 | $Fc$ (kg.) | Mean | 7.77 | 7.81 | 7.33 | 7.24 | 7.22 | 6.62 | 6.37 | 5.67 | 5.15 | 7.98 |
| | | s | 0.620 | 0.668 | 0.598 | 0.496 | 0.476 | 0.434 | 0.482 | 0.613 | 0.525 | 1.034 |
| | $\Delta h$ (cm.) | Mean | 11.6 | 4.8 | 4.8 | 5.0 | 5.3 | 5.5 | 6.0 | 6.7 | 8.1 | 12.5 |
| | | s | 0.12 | 0.16 | 0.18 | 0.18 | 0.18 | 0.17 | 0.17 | 0.17 | 0.18 | 0.17 |
| Cremona 2 | $Fc$ (kg.) | Mean | 8.34 | 7.92 | 7.91 | 7.76 | 7.38 | 7.07 | 6.56 | 6.25 | 5.50 | 8.48 |
| | | s | 0.584 | 0.581 | 0.617 | 0.508 | 0.577 | 0.540 | 0.399 | 0.426 | 0.479 | 0.667 |
| | $\Delta h$ (cm.) | Mean | 10.8 | 4.2 | 4.5 | 4.7 | 4.8 | 5.2 | 5.4 | 6.5 | 7.7 | 11.2 |
| | | s | 0.23 | 0.15 | 0.17 | 0.10 | 0.17 | 0.19 | 0.22 | 0.02 | 0.14 | 0.22 |

C. Flat knot.

| $\alpha$ | | | 0°* | 23° | 35° | 41° | 47° | 53° | 60° | 67° | 74° | 81° | 89° | 97° | 106° | 128° | 180°** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shortening (%) | | | 100 | 80 | 70 | 65 | 60 | 55 | 50 | 45 | 40 | 35 | 30 | 25 | 20 | 10 | 0 |
| Cotton 1 | *Fc* (kg.) | Mean | 8.95 | 8.56 | 8.48 | — | 8.38 | — | 7.98 | — | 7.72 | — | 7.39 | — | 7.22 | 7.00 | 8.45 |
| | | s | 0.572 | 0.551 | 0.488 | — | 0.695 | — | 0.651 | — | 0.578 | — | 0.756 | — | 0.533 | 0.614 | 0.536 |
| | $\Delta h$ (cm.) | Mean | 15.9 | 6.9 | 7.1 | — | 7.3 | — | 7.4 | — | 7.8 | — | 8.5 | — | 9.8 | 12.0 | 15.7 |
| | | s | 0.32 | 0.18 | 0.19 | — | 0.22 | — | 0.21 | — | 0.16 | — | 0.21 | — | 0.21 | 0.22 | 0.35 |
| Cotton 2 | *Fc* (kg.) | Mean | 9.65 | 9.30 | 9.35 | — | 8.95 | — | 8.42 | — | 8.07 | — | 7.92 | — | 7.60 | 7.06 | 9.06 |
| | | s | 0.703 | 0.535 | 0.586 | — | 0.521 | — | 0.499 | — | 0.429 | — | 0.583 | — | 0.361 | 0.413 | 0.544 |
| | $\Delta h$ (cm.) | Mean | 12.0 | 5.1 | 5.4 | — | 5.4 | — | 5.4 | — | 5.6 | — | 6.5 | — | 7.6 | 9.4 | 10.5 |
| | | s | 0.48 | 0.18 | 0.18 | — | 0.18 | — | 0.23 | — | 0.23 | — | 0.23 | — | 0.23 | 0.14 | 0.42 |
| Cremona 1 | *Fc* (kg.) | Mean | 8.44 | 7.97 | 7.70 | 7.62 | 7.28 | 8.07 | 8.48 | 8.91 | 8.93 | 8.68 | 8.44 | 8.00 | 7.20 | 5.99 | 7.73 |
| | | s | 0.668 | 0.533 | 0.544 | 0.579 | 0.386 | 0.489 | 0.569 | 0.498 | 0.540 | 0.515 | 0.583 | 0.679 | 0.701 | 0.601 | 0.992 |
| | $\Delta h$ (cm.) | Mean | 12.1 | 4.7 | 4.8 | 4.9 | 4.9 | 5.2 | 5.4 | 5.7 | 5.9 | 6.1 | 6.5 | 6.6 | 7.0 | 8.3 | 11.5 |
| | | s | 0.14 | 0.19 | 0.16 | 0.20 | 0.11 | 0.15 | 0.14 | 0.10 | 0.17 | 0.14 | 0.17 | 0.15 | 0.19 | 0.17 | 0.15 |
| Cremona 2 | *Fc* (kg.) | Mean | 8.59 | 7.90 | 7.57 | — | 7.34 | — | 8.16 | 8.89 | 8.77 | 8.48 | 8.31 | — | 6.79 | 5.51 | 8.21 |
| | | s | 0.515 | 0.321 | 0.590 | — | 0.476 | — | 0.540 | 0.493 | 0.599 | 0.775 | 0.684 | — | 0.575 | 0.774 | 0.942 |
| | $\Delta h$ (cm.) | Mean | 10.4 | 4.1 | 4.2 | — | 4.3 | — | 4.7 | 5.0 | 5.2 | 5.4 | 5.6 | — | 6.2 | 7.2 | 9.8 |
| | | s | 0.19 | 0.16 | 0.17 | — | 0.14 | — | 0.14 | 0.16 | 0.12 | 0.26 | 0.13 | — | 0.02 | 0.26 | 0.21 |

s : Standard deviation.

* In both the upper and the lower bar, one of the chucks is set in the middle of the bar ($d$=0 cm.), to which a pair of legs represented by a single cord are fastened. Length of each leg $l$ is 25 cm. instead of 10 cm.. Hence, $h$=50 cm..

** The chucks are set as above, and two legs representing different cords are fastened to the chuk. $l$=25 cm. and $h$=50 cm..

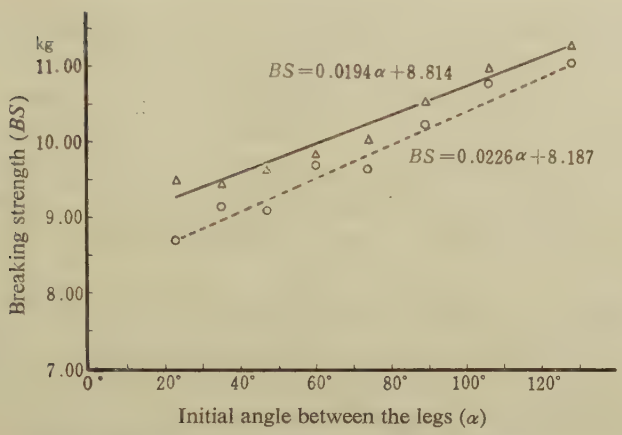



Fig. 4. Relationship between the breaking strength of netting cord and the angle $\alpha$ in the "plain link": Cotton 2 (triangle); Cremona 2 (circle).

It is shown in Fig. 4 that the breaking strengths of the Cotton 2 and Cremona 2 cords decrease in proportion to the decrease in the initial angle between the two legs representing the same piece of cord. It may be said that this result is in accordance with those of TAUTI.

As Table 1 shows, the Cremona 2 cord has greater breaking strength than Cotton 2 cord when both cords are straight. When linked by a "plain link", however, the breaking strength of the former is smaller for the some value of $\alpha$, and is more susceptive of the change of $\alpha$, than that of the latter, as is indicated in Fig. 4.

2) *Breaking strength in the trawler knot.*

The breaking strengths of the four kinds of netting cords tied into the trawler knot are calculated from the data of Table 2B and plotted against the angle $\alpha$ in Fig. 5.

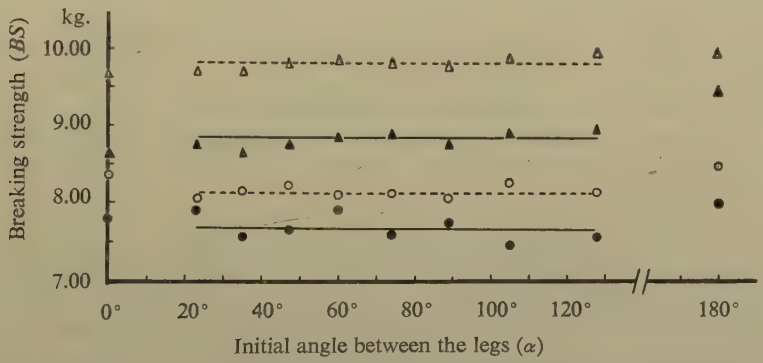



Fig. 5. Relationship between the breaking strength of trawler knot and the angle $\alpha$: Cotton 1 (solid triangle); Cotton 2 (open triangle); Cremona 1 (solid circle); Cremona 2 (open circle).

This figure shows that the breaking strength of each netting cord is nearly independent of $\alpha$ within the range of $\alpha$ from 23° to 128°. In each kind of cord, the mean breaking strength for the range of $\alpha$ from 23° to 128° is defined as $BS_\alpha$ and indicated by a horizontal line in Fig. 5. The breaking strengths for $\alpha=0°$ and $\alpha=180°$ are denoted by $BS_{0°}$ and $BS_{180°}$ respectively. The values of $BS_\alpha$, $BS_{0°}$ and $BS_{180°}$ for the four kinds of netting cord are shown in Table 3. It is seen from this table that in every netting cord $BS_\alpha$ is closer to $BS_{0°}$ than to $BS_{180°}$.

Table 3. Breaking strengths of four kinds of netting cord tied into the trawler knot.

| | $BS_{0°}$ (kg.) | $BS_\alpha$ (kg.) | $BS_{180°}$ (kg.) |
|---|---|---|---|
| Cotton 1 | 8.64 | 8.82 | 9.45 |
| Cotton 2 | 9.72 | 9.81 | 9.95 |
| Cremona 1 | 7.77 | 7.67 | 7.98 |
| Cremona 2 | 8.34 | 8.14 | 8.48 |

3) *Breaking strength in the flat knot.*

The breaking strengths of cotton and cremona cords tied into the flat knot are plotted against the value of $\alpha$ in Fig. 6. It is seen from this figure that the breaking strengths of cotton cords tied into the flat knot are nearly constant for the values

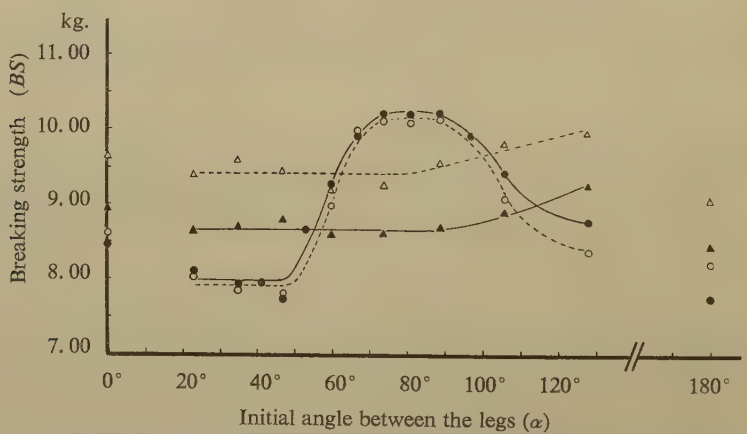



Fig. 6. Relationship between the breaking strength of flat knot and the angle $\alpha$: Cotton 1 (solid triangle); Cotton 2 (open triangle); Cremona 1 (solid circle) Cremona 2 (open circle).

of $\alpha$ from 23° to about 90°, but increase linearly as $\alpha$ increases from about 90° upto 128°. The nearly constant breaking strength of each cotton cord concurrent with the values of $\alpha$ from 23° to about 90° falls between the values of $BS_{0°}$ and $BS_{180°}$ of the respective cord as shown in Table 4. $BS_{0°}$ and $BS_{180°}$ of each kind of cord tied into the flat knot are defined in the same manner as in the trawler knot, and their values are obtained from Table 2C.

Table 4. Breaking strengths of four kinds of netting cord tied into the flat knot.

| | $BS_{0}°$ (kg.) | $BS_{\alpha}$ (kg.) | $BS_{180}°$ (kg.) |
|---|---|---|---|
| Cotton 1 | 8.95 | 8.67 | 8.45 |
| Cotton 2 | 9.65 | 9.41 | 9.06 |
| Cremona 1 | 8.44 | 10.18 | 7.73 |
| Cremona 2 | 8.59 | 10.09 | 8.21 |

As is clearly seen in Fig. 6, the breaking strength of each cremona cord tied into the flat knot changes in relation to the angle $\alpha$ in a rather complex manner; it remains nearly constant for the smaller values of $\alpha$ (23°–47°), increases in the range of $\alpha$ from ca. 50° to ca. 70°, shows the maximum throughout the range of $\alpha$ between ca. 70° and ca. 90° and decreases as $\alpha$ increases from ca. 90° to 128°. The maximum value is greater than either $BS_{0}°$ or $BS_{180}°$ of the respective cord as shown in Table 4. In this table, $BS_{\alpha}$ is the mean breaking strength for the range of $\alpha$ from 23° to 89° in cotton cords and from 74° to 89° in cremona cords.

The reason why the breaking strength of a netting cord, either of cotton or of cremona, tied into a flat knot changes in a peculiar manner in relation to $\alpha$ as compared with that of a cord tied into a trawler knot or connected by plain link can be explained from the fact that a flat knot is deformed in different ways according to the direction in which its legs are pulled (Fig. 7).

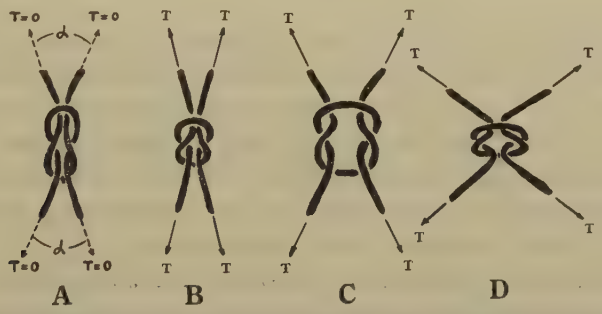



Fig. 7. Deformation of a flat knot due to the static load applied to its legs. A, Initial shape; B, Tightening that occurs when the angle $\alpha$ is small; C, Loosening that occurs when the angle $\alpha$ is large; D, Flattening that occurs after loosening where $\alpha$ is ca. 105°–128°.

If a pair of legs of a flat knot that represent a single cord contain a small angle between them and are pulled gradually by a strong force, the knot becomes tightened and its size is reduced (Fig. 7B), until one of the leg is broken at the knot. It is easy to see that the radius of curvature of the cord is reduced when the knot is tightened in this way. The flat knot made of the Cotton 1 or 2 cord undergoes this type of deformation when $\alpha$ is between 23° and ca. 75°, and the one made of the Cremona 1 or 2 cord does so when $\alpha$ is between 23° and ca. 50°. It is exactly within

these ranges of $\alpha$ that the breaking strength of the flat knot made of the respective cord remains nearly constant (Fig. 6). When $\alpha$ is between 60° and ca. 75°, however, the flat knot made of cotton cords is once loosened in the manner shown in Fig. 7**C** in most cases and then tightened as shown in Fig. 7**B** before breakage, while it is directly tightened in the other cases.

On the other hand, if a pair of legs representing a single cord are pulled containing a large angle between them (ca. 75°–128° in the case of Cotton 1 and 2, and ca. 50°–ca. 105° in the case of Cremona 1 and 2), the knot is loosened in the manner shown in Fig. 7**C** and a leg is broken at the knot. When $\alpha$ is as large as 106° or 128°, the knot is once loosened as in Fig. 7**C** and flattened later in the manner indicated in Fig. 7**D** and then one of the legs is broken at the knot. It may be said that the radius of curvature of the netting cord increases when a flat knot is loosened. When the angle $\alpha$ increases beyond 90° (in cotton cords) or 5o° (in Cremona cords), the breaking strength of the flat knot shows either a gradual increase (in the case of Cotton 1 and 2) or an increase followed by a leveling and a consecutive decrease (in the case of Cremona 1 and 2).

When a pair of legs of a trawler knot are pulled gradually by a strong force, the knot is always tightened in a manner similar to Fig. 7**B** and never undergoes the deformation indicated in Fig. 7**C** or **D** irrespective of the direction of the pull. This is probably the reason why the breaking strength of netting cord tied into a trawler knot is not affected by the value of $\alpha$.

## SUMMARY

In order to clarify the effect of the spreading of the mesh to the breaking strength of the netting cord, two pieces of the cotton or cremona netting cord were linked by a "plain link" or tied into a flat knot or a trawler knot, and the breaking strength of the cord was investigated by changing the angle between the neighboring legs of the knot and applying static loads to the legs. The cord was broken almost always at the knot or crossing point, and only the breaking strengths of such cases were treated with. Following results were obtained:

1) In the plain link, the breaking strength of the netting cord is affected by the angle between the neighboring cords. The relation between the breaking strength and this angle is linear; the breaking strength increases as the angle $\alpha$ (i.e., the angle between a pair of legs representing a single piece of cord) increases. The effect of this angle to the breaking strength is greater in the cremona cords than in the cotton cords.

2) The breakinng strength of the netting cord tied into the trawler knot is hardly affected by the angle $\alpha$ throughout the range of $\alpha$ from 23° to 128°, and the value is closer to the breaking strength of the mesh stretched longitudinally (i.e., in the ordinary direction) than to that of the mesh stretched transversely (laterally). This holds in cotton cords as well as in cremona cords.

3) The breaking strength of the cotton cord tied into the flat knot is almost constant when the angle $\alpha$ is between 23° and about 90° and this value falls between

the breaking strength of the mesh stretched longitudinally and that of the mesh stretched transversely.

4) The breaking strength of the cremona cord tied into the flat knot varies with the angle $\alpha$. When this angle is between ca. 70° and ca. 90°, the breaking strength is at the maximum and is greater than in the mesh fully stretched either longitudinally or transversely. When the angle is between 23° and about 50°, the breaking strength is comparatively small and nearly constant, and falls between the breaking strength of the mesh stretched longitudinally and that of the mesh stretched transversely.

Acknowledgement:

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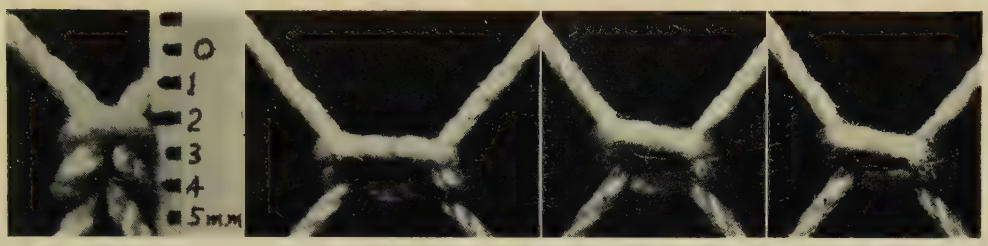
## EXPLANATION OF PLATES

Plates I and II. Successive stages of deformation of the flat knots when static load ($F$) is applied to the legs as shown in the following table.

| Plate | Fig. | Cord | $\alpha$ | A | | B | | C | | D | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $F$ (kg.) | $T$ (kg.) | $F$ (kg.) | $T$ (kg.) | $F$ (kg.) | $T$ (kg.) | $F$ (kg.) | $T$ (kg.) |
| I | 1 | Cotton 1 | 89° | 0 | 0 | 1.5 | 0.9 | 3.0 | 1.8 | 4.9 | 2.9 |
| | 2 | Cremona 1 | 89° | 0 | 0 | 3.2 | 2.0 | 7.1 | 4.2 | — | — |
| | 3 | Cotton 1 | 74° | 0 | 0 | 2.2 | 1.3 | 4.8 | 2.7 | 7.6 | 4.3 |
| | 4 | Cremona 1 | 74° | 0 | 0 | 2.2 | 1.3 | 4.4 | 2.5 | 6.3 | 3.6 |
| II | 5 | Cotton 1 | 60° | 0 | 0 | 4.0 | 2.1 | 6.5 | 3.5 | — | — |
| | 6 | Cremona 1 | 60° | 0 | 0 | 3.0 | 1.6 | 6.0 | 3.3 | 7.8 | 4.2 |
| | 7 | Cotton 1 | 47° | 0 | 0 | 2.9 | 1.5 | 4.8 | 2.5 | 6.1 | 3.2 |
| | 8 | Cremona 1 | 47° | 0 | 0 | 4.0 | 2.1 | 5.9 | 3.1 | 7.9 | 4.1 |

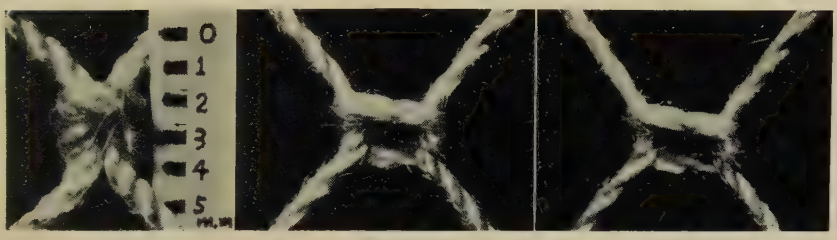
$F$ : Static load.
$T$ : Tension of the leg.
$\alpha$ : Initial angle between the legs.

Fig. 1



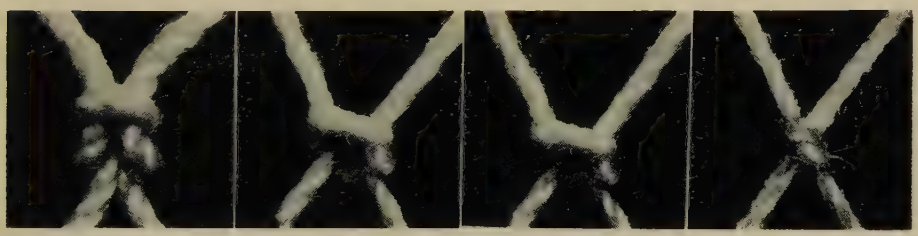

A B C D

Fig. 2



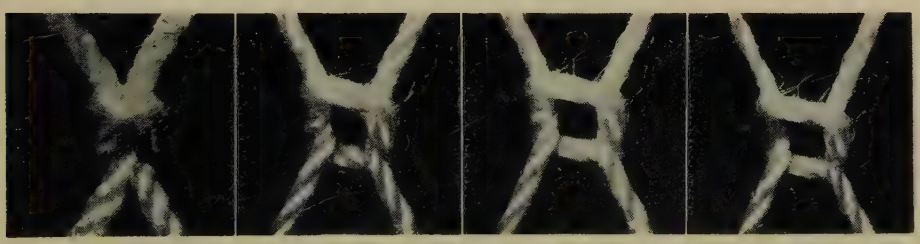

A B C

Fig. 3



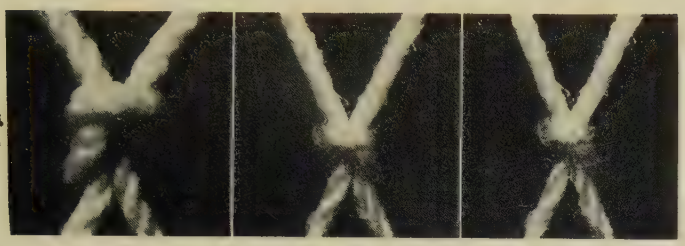
A B C D

Fig. 4



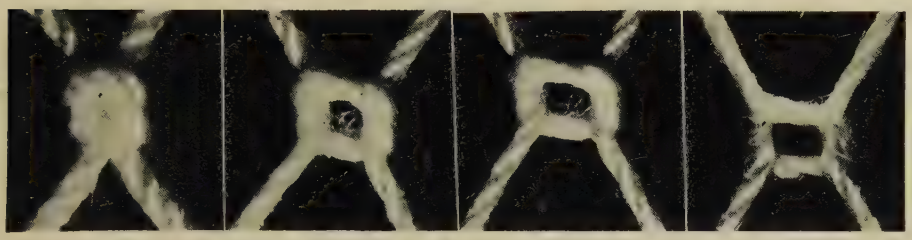
A B C D

Fig. 5



A B C

Fig. 6



A B C D

Fig. 7

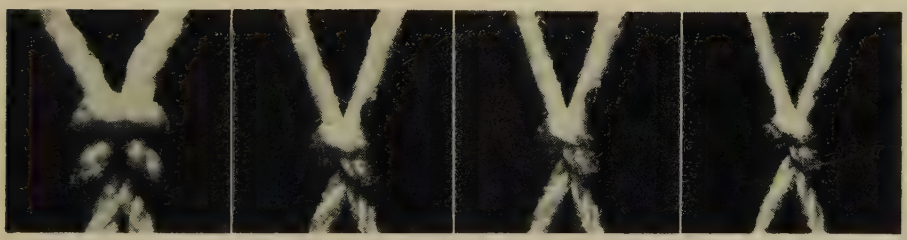


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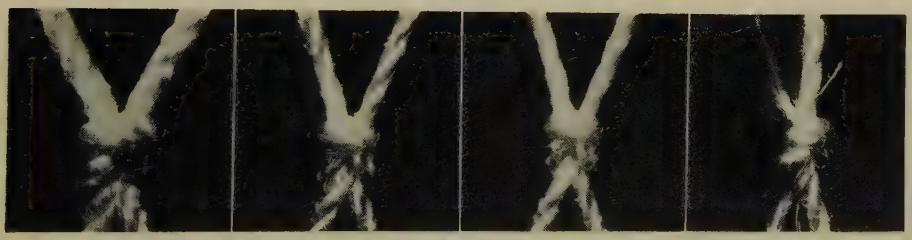
Fig. 8



A B C D

# 福山港水域の海況の概要

松平康雄・小山治行・遠藤拓郎
（広島大学水畜産学部水産学科）

# Hydrographic Conditions of Fukuyama Habor

Yasuo MATSUDAIRA, Haruyuki KOYAMA & Takuro ENDO
*Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Text-figs. 1–14; Tables 1–7; Appendix Tables A–F)

(I) まえがき …… 247
(II) 調査水域の地形 …… 248
(III) 観測方法 …… 250
(IV) 観測結果および考察 …… 251
1. 海況 …… 252
2. 底質 …… 270
(V) 論議 …… 275
1. 福山港水域の基本的特長 …… 275
2. 生物群集および漁業 …… 278
3. 観測方法について …… 281
(VI) 要約 …… 281
(VII) Summary …… 282
(VIII) 引用文献 …… 284
Appendix: Data of hydrographic observations (Tables A–F) …… 286

## (I) まえがき

沿岸漁業・水産養殖業の漁場として重要ないわゆる“浅海”“内湾”においては，海況の変化，物質循環の様相が外洋よりはるかに複雑であるが，これらの変化・様相を明らかにすることは学問的に興味深く，水産の見地からも重要なことと考えられる．

われわれは1958～60年に広島県福山港水域で，潮汐による海況の変化，底質の有機質含量などにつき調査*したので，その結果を報告する．

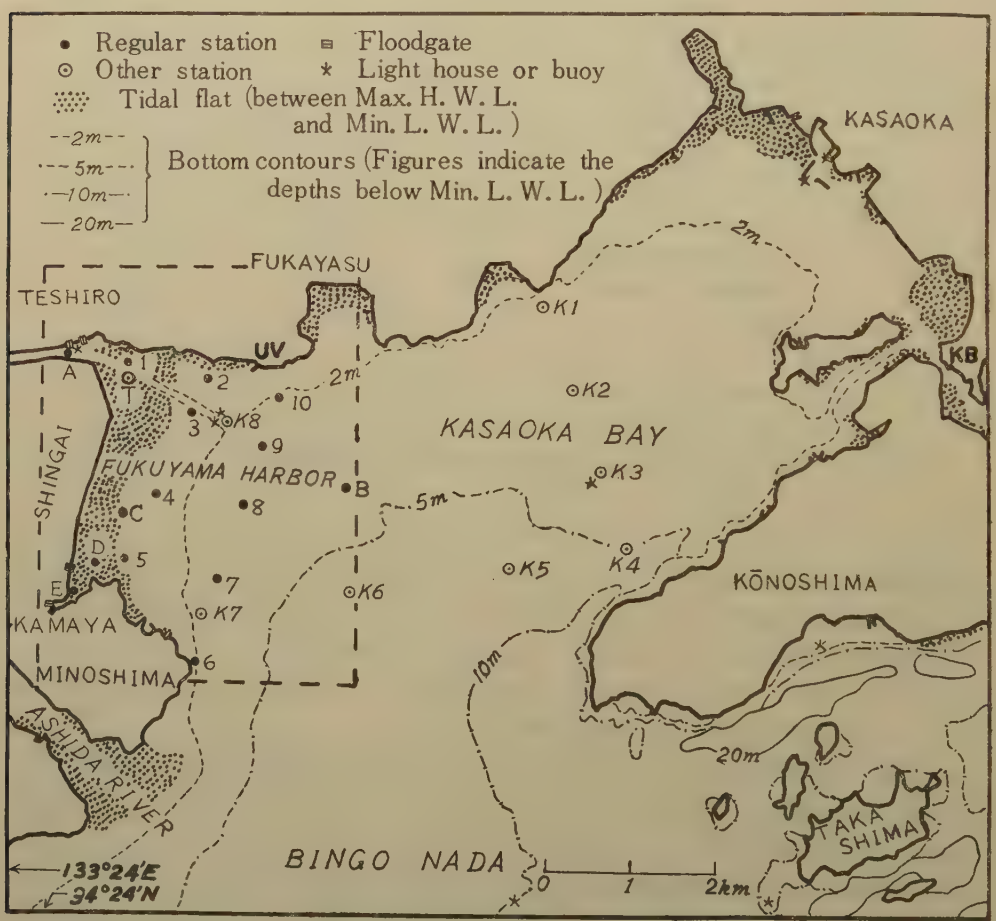
この水域は当学部の敷地に接し，教官・学生の実験・研究が頻繁に行なわれる場所であるが，従来その海況全般を記述した資料がなく不便を感じていたので，本報告では上記の調査結果のほかに既刊の各種のデータを援用して，この水域の海域を概説した．

本文に入るに先立ち，当学部観測艇“かもめ”を運転して観測に協力された神田富夫氏，観測の一部に助力された水産学科第6回卒業生弓場登・岡山武・山本富一の諸氏，観測結果の整理に協力された弓場幹子夫人・藤井諏恵子嬢に厚く感謝する．また降水量・河川流量の資料入手に便宜を賜わった松永測候所および福山市水道局に深謝する．

* 主として昭和33, 34年度農林省農林漁業試験研究費によった．

## (II) 調査水域の地形

調査を行なった福山港水域*は，備後灘東部に開く笠岡湾の西部を占め (Text-fig. 1)，北・西・南の3方を陸にかこまれた台形状の湾入部である．台形の上辺にあたる西岸は約 3km (直線距離，以下同じ)，両側辺にあたる南・北岸はいずれも約 2km，下辺にあたる東側の開口部 (St. 6～10 を通る線) は約 4km で，この 4 辺にかこまれる水域の面積は約 6.1km$^2$ である．南・北岸は標高 130m 以下の丘陵 (石英班岩質) から成り樹林・畑地でおおわれ，多少の凹凸を示す海岸線の凹部には小部落が発達している．西岸は平坦な干拓農地で，護岸堤防以外は標高が平均海面下 0.5m 内外，海岸線は直線状の護岸堤防から成り，その北端に福山入江，南端に釜屋入江が開口する．




Text-fig. 1. General map of the investigated area. (After Chart No. 1118[10])
UV Faculty Campus
KB Former site of the Kasaoka Branch of the Naikai Regional Fisheries Research Laboratory.
The area enclosded with the thick broken line is reproduced in Text-figs. 4, 5, 7, 8 and 12.

---

* 調査水域は行政上の“福山港域”(ほぼ St. 5, 9 を通る線以北の海面) とその隣接水域とを含むが，本報では両者をあわせて“福山港水域”と呼ぶことにする．

水深は浅く，最も沖側の St. B でも基準面下4.5m（大潮満潮面下約8.1m）にすぎず，西岸ぞいのかなりの部分が大潮干潮時に干出する．海底は平坦で西から東へゆるやかに傾斜し，底質は軟泥質である．調査水域の東北部には，福山入江口からほぼ SE/E にのびる浚渫航路があり（延長1.7km，巾100m，深さ基準面下2～3m），2m等深線に達する．

流入河川は，顕著なものはなく，陸水は主として福山・釜屋両入江およびその入口付近にある農地排水樋門を経て本水域に流入する．福山入江は延長約 3km の機帆船用水路で，巾約100m，深さ1.5m内外（基準面下），本水域の東北隅から西方にのび，その西端付近が福山港の埠頭になっており，ここに福山市の都市・工場廃水のかなりの部分が流入し，また海に至る間で農地排水が流入する．これらの陸水は，入江の下層を占める海水と次第に混合しつつ入江口に達する[16]．農地排水樋門は福山入江口北岸に2カ所，釜屋入江奥に2カ所，同入江北岸に1カ所あり（Text-fig. 1），干潮時に開かれて背後の干拓農地の余剰水を排出する．この地方は Table 1 に見るように降水量が少ないため，月間降水量の比較的多い（100～200 mm）4～10月以外は，上記の経路を経て調査水域に流入する陸水の量はわずかである．

Table 1. Monthly precipitation at Matsunaga*, Hiroshima Pref.

| Year | Precipitation (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sept. | Oct. | Nov. | Dec. | Annual total |
| 1942~'57 (Mean) | 39.0 | 52.4 | 86.5 | 97.2 | 112.6 | 179.5 | 204.5 | 67.9 | 173.8 | 99.8 | 60.3 | 36.7 | 1210.2 |
| 1958 | 55.9 | 48.3 | 100.4 | 166.8 | 65.0 | 170.0 | 231.8 | 139.3 | **72.3** | 99.2 | **30.2** | 47.1 | 1226.3 |
| 1959 | 58.0 | 110.0 | **80.7** | 98.9 | 137.4 | 68.5 | **148.2** | 178.3 | 81.3 | 86.5 | 59.0 | 66.2 | 1173.0 |
| 1960 | 28.0 | 5.7 | 78.8 | 72.2 | 102.5 | 146.2 | **245.3** | 138.2 | 148.4 | 79.7 | 52.9 | 8.1 | 1106.0 |

* Located ca. 16km west of the Faculty Campus.
**Gothic type** indicates the months in which the hydrographic observation was conducted.
Source : Matsunaga Weather Station.

なお，調査水域の西南 1.5km に芦田川が放流しており，その河水の影響が調査水域の南部におよぶ場合が多く，降水量が比較的多く偏南風の卓越する暖季にこの傾向が強い．芦田川の放水量の季節変化を Table 2 に示した．

Table 2. Average daily flow of the Ashida River*
(Unit : 1,000m³ per day)

| 1960 | | 1961 | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sept. | Oct. | |
| 427 | 355 | 352 | 433 | 550 | 749 | 644 | 284 | 1350 | 244 | 916 | 930 | 605 |

* Measured at Kami-yamamori, 16km from the mouth of the river.
Source : Water Supply Department, Fukuyama Municipal Office.

調査水域の顕著な特長のひとつは，潮差が大きいことである．これは，豊後・紀伊の両水道から瀬戸内海へ進入する2潮浪がこの近海（備後灘東部）で出会うためで，調査海域の大

潮時の潮差は平均3.0mに達する．ただし日潮不等は著しくない．福山港（福山入江奥）・笠岡港・鞆港の潮汐非調和常数を Table 3 に示した．

Table 3. Non-harmonic tidal constants at Fukuyama, Kasaoka and Tomo Ports.

| Port | Fukuyama | Kasaoka | Tomo |
|---|---|---|---|
| M. H. W. I. | $11^h19^m$ | $11^h21^m$ | $11^h17^m$ |
| Mean sea level | 2 2 | 2 08 | 2 11 |
| Spring rise | 3 8 | 3 60 | 3 61 |
| Neap rise | 2 9 | 2 74 | 2 77 |
| Spring range | (No data) | 3 04 | 3 00 |
| Neap range | (No data) | 1 32 | 1 32 |
| Mean range | (No data) | 2 18 | 2 16 |
| Diurnal inequality | (No data) | 0 37 | 0 41 |
| Source | Maritime Safety Agency[11] | Matsudaira & Yoshizawa[19] | |

## (III) 観 測 方 法

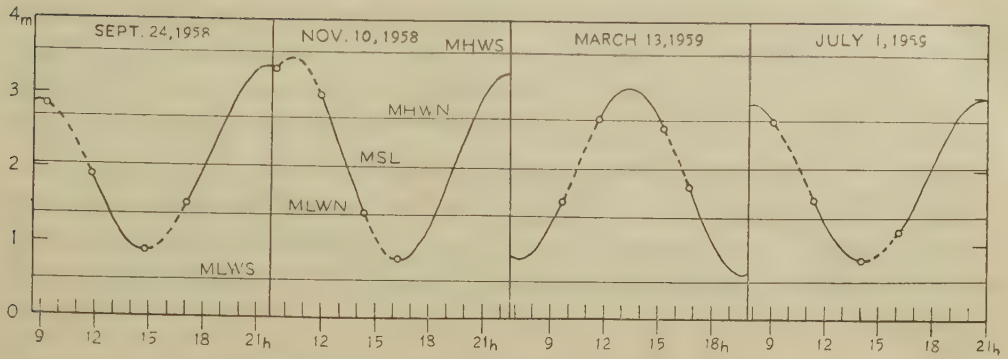
### 1. 海 況

海況観測は合計6回行なった (Table 4)．"定期観測 I ～IV" は潮時による海況の変化を各季節に調査したもので，風波のない日の午前・午後に各1回，同じ10～14の観測点を1周して観測した．1周には約2時間を要した．午前の観測はおよそ満潮時に，午後の観測はおよそ干潮時になるように計画したが，"定期観測III" では午前は上げ潮時，午後は下げ潮時となった (Text-fig. 2)．"12時間観測" では福山入江口付近の水の流動交代を調査し，"流入陸水調査" では数日前の大雨にともなう流入陸水の分布状態を調査した．

Table 4. List of hydrographic observations.

| Observation | Date (Moon age) | Time | | Stations occupied (No. of stations) |
|---|---|---|---|---|
| Regular Observation I | 24/IX/1958 (11) | $09^h12^m$～$11^h42^m$<br>14 45 ～16 59 | *(H+1)～(H+3)<br>(L) ～(L+2) | St. 1～10 (10) |
| Regular Observation II | 10/XI/1958 (28) | 09 32 ～11 58<br>14 21 ～16 15 | (H−1)～(H+1)<br>(L−2)～ (L) | St. 1～10, A～E (14) |
| Regular Observation III | 13/III/1959 (4) | 09 38 ～11 39<br>15 16 ～16 38 | (L+2)～(L+4)<br>(H+2)～(H+3) | St. 1～10 (10) |
| Regular Observation IV | 1/VII/1959 (25) | 09 03 ～11 20<br>14 00 ～16 12 | (H+1.5)～(H+3.5)<br>(L) ～(L+2) | St. 1～10, A, B (12) |
| 12-Hour Observation | 10/VII/1960 (16) | 05 30 ～17 30 | (L)～(H)～(L) | St. T (1) |
| Land Drainage Survey | 11/VII/1960 (17) | 11 05 ～12 45 | (H−1)～(H+0.5) | St. K1～K8 (8) |

* (H) and (L) respectively represent high water and low water. Numerals indicate the length of time in hours. Consequently, (H+1), (L−2), etc. respectively denote "one hour after high water", "two hours before low water", etc.




Text-fig. 2. Predicted tide curves at Minoshima for the days of regular observations. Broken part of the curve indicates the duration of observation.

観測層は原則として0m層，1m層，および底層（海底上0.3～1.5m）とし，観測項目はAppendix Table A～Fの通りである．採水・測温には北原式B号採水器・棒状温度計（ときにナンセン採水器・転倒温度計）を用い，必要に応じサーミスター水温計を併用した．透明度板は直径30cmの白色のもの，表層潮流の測定には十字海流板（各面30×30cm）を用いた．pHは比色法（指示薬：cresol redまたはthymol blue），塩素量・溶存酸素量は“海洋観測指針”[24]の常法，C.O.D.は佐伯[30]のアルカリ性過マンガン酸カリ法（ただしN/50 $KMnO_4$を5ml使用）によった．酸可溶性全鉄量は$\alpha\alpha'$-dipyridylで発色させ比色[23]したが，浸出時間を30分とし還元液には10% $Na_2SO_3$を用いた．

**2. 底　　質**

底質試料はSt. 1～10で次の6回採取した．

〔I〕 1958年10月3日
〔II〕 〃 11月7日
〔III〕 〃 12月22日
〔IV〕 1959年1月31日
〔V〕 〃 3月13日
〔VI〕 〃 7月1日（St. Aを追加）

採泥にはT型採泥器を用い，同時に古川[4]の浅海用ペネトロメーターを水面から落下させて貫入深度*（penetration value）を記録した．底泥の酸素消費量の測定には，採泥直後の湿泥5gを250ml容のガラス共栓瓶（無色）に入れ，溶存酸素量既知の海水を満たして密栓し，振とうして内容物をよく混合し，24時間室温に静置した後，瓶内の上澄液の溶存酸素量をWINKLER法で測定した．灼熱減量は常法[32]，有機炭素量はクロム硫酸を用いるTiurinの簡易滴定法[32]，全窒素量はmicro-KJELDAHL法により測定した．

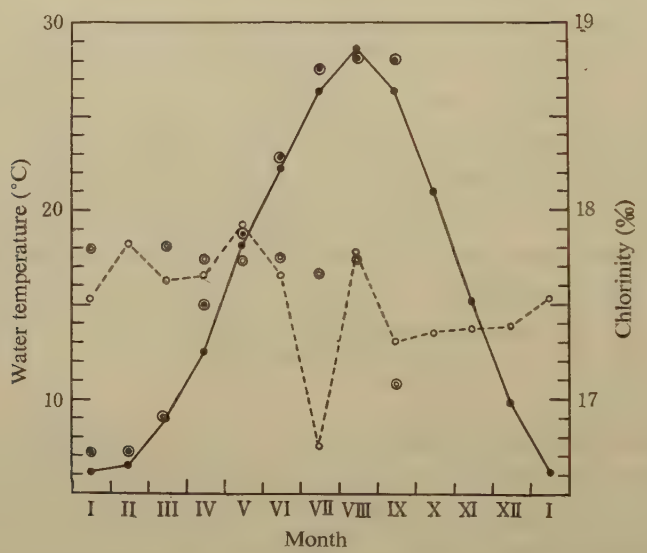
## (IV)　観測結果および考察

上記の海況観測の結果をAppendix Table A～Fに，底質に関する測定結果をTable 6に示した．

これらの観測結果を考察する前に，調査水域の水温・塩素量の年変化にふれておこう．Text-

* 古川[4]は“透徹深度”と呼んでいる．

fig. 3 に学部前* および内海区水産研究所笠岡支所前** の表層の水温・塩素量の月別平均値を示した．前者は1961年1～9月の記録しかないが，後者は1939～53年の15年間の平均値である．各季節に大学前と笠岡支所前とでどの程度の差があるかはまだ明かでないが，かりにこの差があまり大きくないとみなせば，この図から，学部前の表層水温は1,2月に最低（月平均6～7°），8月に最高（同28°位）に達し，表層塩素量は2～6月には17.6～17.9‰，7～9月には流入陸水の影響で低下し，10～1月の間に17.5‰前後にまで回復するものと考えられる．すなわち，水温は年較差が大きく最高最低が気温のそれと同月に起るという内湾的な型を示すのに対し，塩素量は流入陸水の多い夏季に低下する以外は周年比較的安定しているといえよう．




Text-fig. 3. Monthly meas of water temperature and chlorinity of the surface layer of Kasaoka Bay.

—•— Water temperature
····○···· Chlorinity
Based on the daily observation at the Kasaoka Branch, Naikai Regional Fisheries Research Laboratory during 1939-1953[10].

⊙ Water temperature
◎ Chlorinity
Based on the daily observation on the south shore of the Faculty Campus in 1961.

## 1. 海　　況

6回の海況観測の結果を順を追って考察する．

(1) 定期観測 I (1958年9月24日)

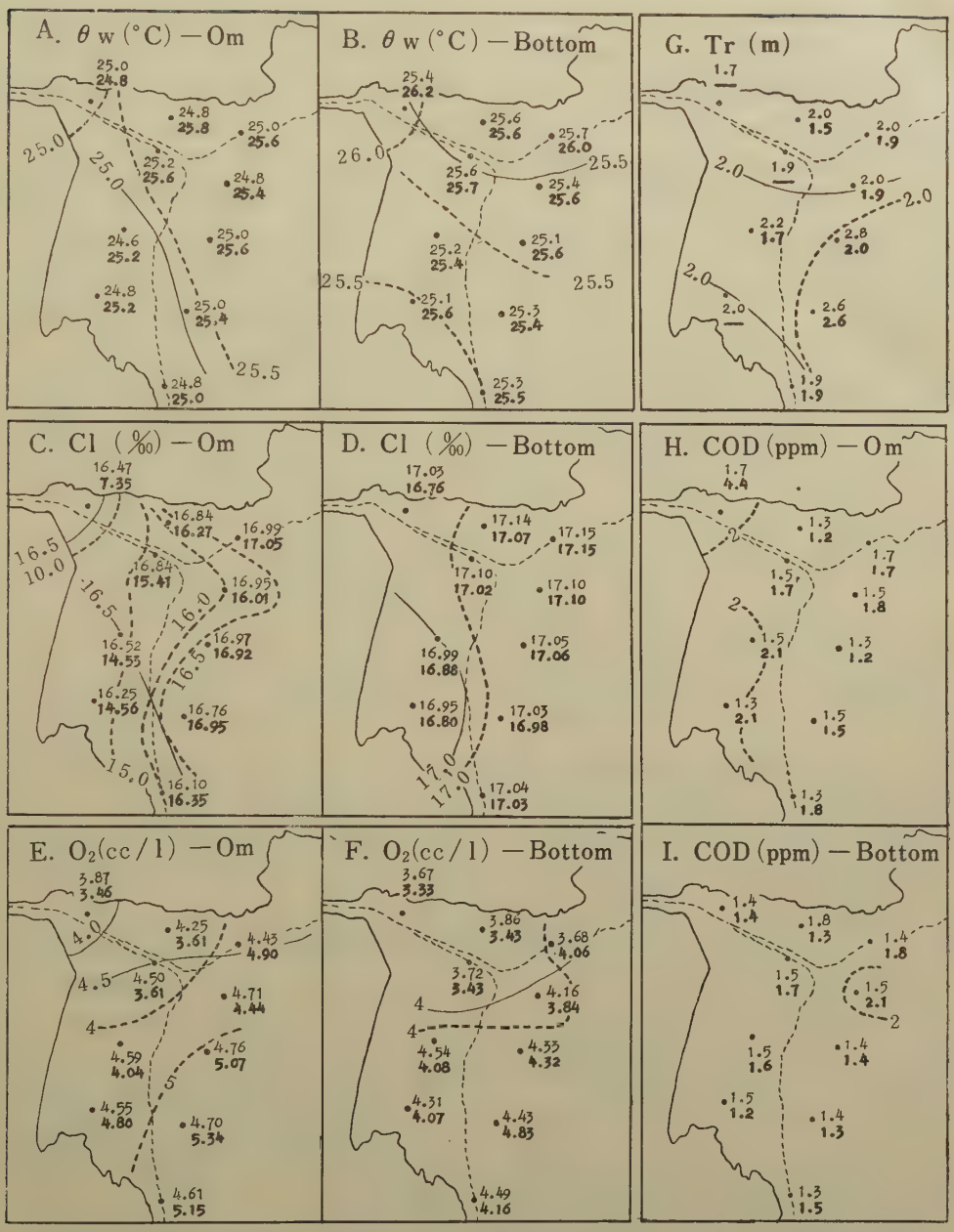
観測結果は Table A-1, A-2 の通りで，その一部を Text-fig. 4 に図示した．

この観測は盛夏の水温最高期がすぎて水温が下り始めた頃に実施したもので，午前の観測は下げ潮の前半に，午後の観測は干潮直後に行なった (Table 4, Text-fig. 2). 天候はくもり (雲量9)，静穏 (北寄りの風 0～2m/sec)，海上の気温は 22～25.8° で表層水温より 1～3° 低い．

---

* 学部敷地南岸のコンクリート・スロープで毎朝9時に観測．塩素量は滴定による．

** 岡山県笠岡市横島 (学部の東方的 8km) にあった (Text-fig. 1)．観測は同支所南岸 (横島水道) で毎朝10時に行なわれ，塩素量は比重から換算された．




Text-fig. 4. Horizontal distribution of water temperature ($\theta_w$), chlorinity (Cl), dissolved oxygen ($O_2$), chemical oxygen demand (C.O.D.) and transparency (Tr) in the two observations on September 24, 1958.
Light-faced letter and solid line ····· Early ebb in the morning
Thick letter and dotted line ····· Early flood in the afternoon

水温 (Text-fig. 4A, B)： 午前午後の各点各深度を通じ 24.6～26.2° の範囲であった．表層は大気により冷却され 1m 層より 0.1～1.0° 低温であるが，1m 層と底層（海底上 1.0～

1.5m) との間ではほとんど差がない．各点とも午後には午前よりわずかに昇温した（表層で約0.5°，底層で約0.2°）．同一深度における水平方向の温度差は1.0°以内で，北寄りのSt. 2, 10がやや高温，南西寄りのSt. 4～6がやや低温であった．学部南岸に近いSt. 10の表層水温は午前25.0°，午後25.6°で，内水研笠岡支所の平年値とほぼ一致する．Text-fig. 3において9月の学部前の水温が笠岡支所前の同月の平年値より2°ちかく高いが，これは恐らく1961年9月が異常に高気温であったことによるもので，一般に9月には学部前と笠岡支所前の表層水温はあまり差がないものと考えられる．

塩素量 (Text-fig. 4 C,D)： 全般的に見て沖側 (St. 8～10) が高く，陸水の流入口に近い岸側が低い．しかし，陸水の影響はほとんど表層に限られ，1m以深の塩素量は入江口付近と沖側とで約0.3‰の差があるにすぎない．底層の塩素量は1m層よりわずかに高い．潮汐による表層塩素量の変化をみると，下げ潮前期には全般に高く，最も低い福山入江口 (St. 1) および南西部 (St. 4, 5) でも16.2～16.5‰である．これに対して，上げ潮初期には，西岸から1.2km沖まで表層一帯に陸水の混入した低塩分水 (14～16‰ Cl) が分布し，下げ潮前期にくらべ塩素量が1～2‰低下した．特に福山入江口では樋門から放出された淡水のために表層の塩素量は7.35‰に低下した．

なお箕島東岸のSt. 6では表層の塩素量が弧立的に低く，芦田川々口の低塩分水がここまで拡がっていたものと推定される．学部南岸付近のSt. 10の表層は17.0‰前後で，さきにのべた季節変化の傾向 (Text-fig. 3) と一致する．

鉛直安定度*： 水温・塩素量の鉛直分布について考察した諸点は，鉛直安定度の値によく現われている．すなわち，下げ潮前期には，0—1m間は大部分の測点で $E' \leqq 0$ で対流状態であったことが裏書きされ，1m以深は一般に $0 < E' < 0.3$ であった．上げ潮初期には，低塩分水が表層をおおった西岸寄りの各点 (St. 1～5) およびSt. 9では0—1m間の $E'$ は1～12で大きい．しかし1m以深は各点とも $0 < E' < 0.3$ で下げ潮前期と大差がない．

溶存酸素量 (Text-fig. 4E, F)： 分布はかなり複雑であるが，福山入江口付近は潮時にかかわらず酸素量が少ない．先ず下げ潮前期（午前）には，福山入江口 (St. 1) の全層および近接するSt. 2, 3の底層が3.8cc/L（飽和度76%）前後の低値を示すが，他の測点では表層は4.2～4.8cc/L (84～94%)，底層はこれよりやや少なく，4.1～4.5cc/L (83～90%) であった．上げ潮初期（午後）には福山入江口付近は表・底層ともさらに低酸素になったが，南方沖寄りのSt. 6～8の表層は逆に酸素量が増し過飽和となった．

本海域のような浅海の溶存酸素量は，アジモ・植物プランクトンの光合成作用，生物・浮泥・底泥による消費，貧酸素（例えば福山入江）または富酸素の水の流入など，諸種の要因によって変化する．今回はこれらの要因を個々に測定しなかったので，観測結果に見られる溶存酸素量の変動の原因を充分に説明することは困難である．

C. O. D. (Text-fig. 4H, I)： 分布および潮時による変化は溶存酸素と同様やや複雑である．顕著なことは，干潮時に福山入江口表層が4.4ppmの高値を示したことである．これに次ぐ高値はSt. 4, 5の表層およびSt. 9の底層（いずれも干潮時）の2.1ppmで，その他は午前・午後を通じて0.9～1.8ppmの範囲であった．潮時による変化および鉛直分布につ

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* 鉛直安定度 $E' = \frac{\sigma_t(z_2) - \sigma_t(z_1)}{z_2 - z_1}$ ただし $z_1$, $z_2$ は観測深度 (m単位)，$\sigma_t(z_2)$, $\sigma_t(z_1)$ はその深度の $\sigma_t$.

いては特別な法則性を見出せなかった．

酸可溶性鉄：　流入陸水や底泥の影響が少ないと考えられる水層では 0.05ppm またはそれ以下であった．下げ潮前期には St. 1 を除くほとんど全点の 0, 1m 層がこれに該当し，上げ潮初期には沖側の St. 6～8, 10 の 0, 1m層がこの値を示した．底層は一般に 0, 1m 層より高値を示し，その最高は 0.23ppm であった．上げ潮初期，低塩分水の分布する St. 1, 3～5 の表層は 0.12～0.38ppm の高値を示した．

透明度 (Text-fig.4G)：　陸岸から最も離れた St. 7, 8 で最も大きく（下げ潮前期にそれぞれ 2.6, 2.8m), 陸岸へ近ずくにつれて減少する傾向が見られた．また大部分の測点で，上げ潮初期には下げ潮前期より透明度が小さかった．

水　　色：　一般に下げ潮前期には5，上げ潮初期には6～7であったが，入江口に近い St. 1, 5 の水色は これより悪かった．また，沖側の測点で下げ潮前期と上げ潮初期を比較すると，塩素量はほとんど変化していないのに，水色は後者の方が明らかに悪い．

総　　括：　以上を総括すると，

① 塩素量の低い季節にあたる．大気による冷却が始まっており，午前の満潮時には 1m 以浅は対流状態を示す．1 m 以深は，塩素量は下方へ向ってわずかに増加するが水温はほとんど変化なく，従って鉛直安定度は大きくない．

② 干潮時には，福山入江・釜屋入江付近から放出される淡水・低塩分水のために，西岸から約 1・2km 沖まで表層の塩素量が満潮時に比し 1～2‰ 低下する．しかしそれより沖側および 1 m 以深はほとんど影響を受けない．次の上げ潮・満潮の間にこの表層の低塩分水は下方の海水と混合してしまう．

③ 沖側やや南寄り (St. 7, 8) には水温・塩素量・溶存酸素量・透明度が比較的高く，水色がよく，C.O.D., 鉄量の小さい水が分布する．これに対し，福山入江の水は低塩分・貧酸素で透明度が小さく C.O.D.・鉄量が大きく，この水の影響は干潮時に浚渫航路周辺におよぶ．南西部 (St. 4, 5) は 水温・塩素量・透明度が 低く，水の交代が比較的悪いと考えられ，干潮時にその表層は釜屋入江付近から放出される陸水の影響を受ける．塩素量・透明度から見て，箕島東岸の St. 6 は芦田川々口の低塩分水の周縁にあたっていたと考えられる．

④ 溶存酸素量は，福山入江水に影響されない水域では，大体 4.1～4.8cc/L（飽和度 83～90%）で，午後表層が過飽和を示す測点もあった．鉛直傾度は著しくなかった．

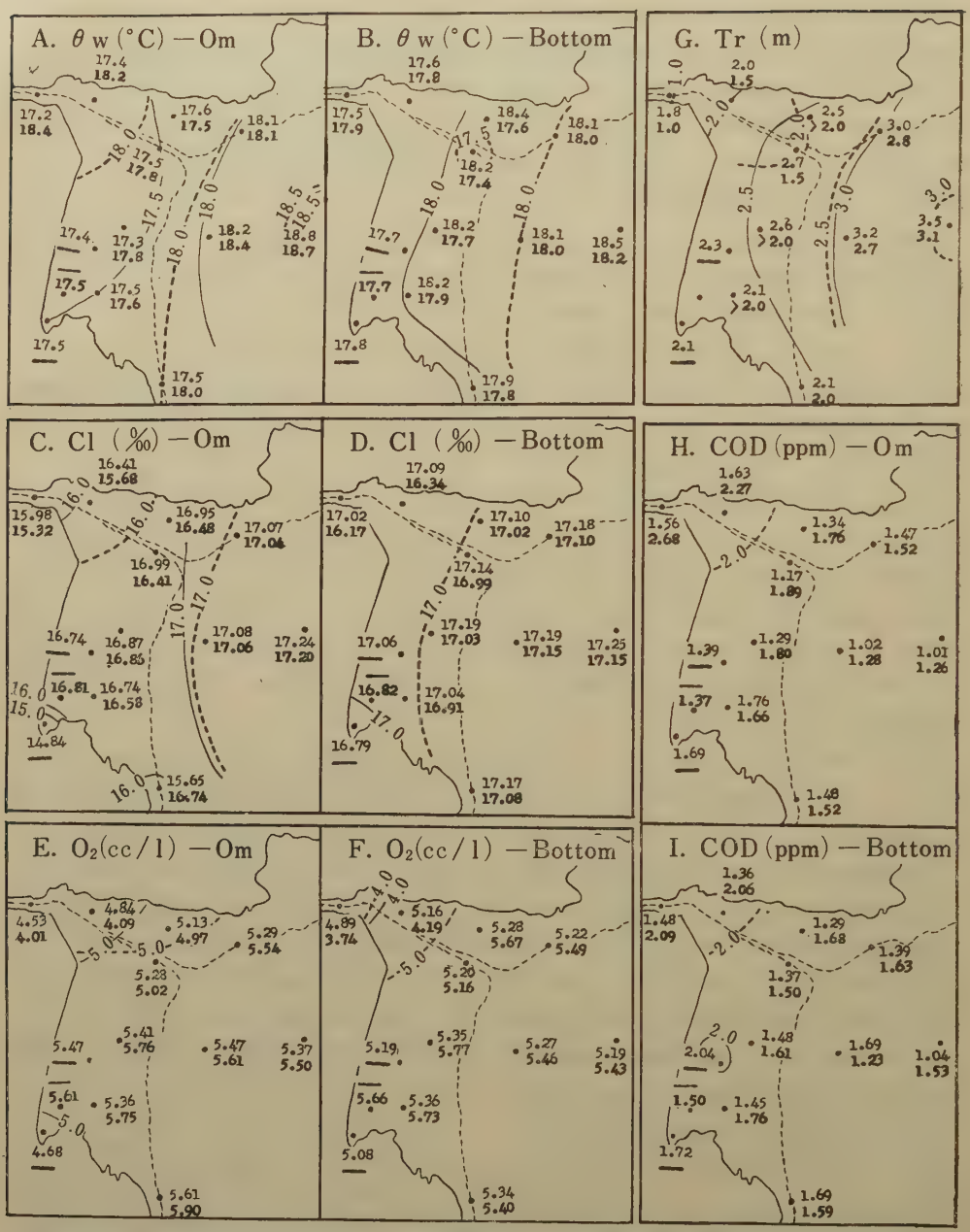
⑤ C.O.D. および酸可溶性鉄は陸水（ことに福山入江水）の混入により増大し，また底層水は中層水に比し一般に鉄の含量が大きい．

(2)　定期観測 II (1958年11月10日)

観測データは Table B-1, B-2 の通りで，おもな要素の水平・鉛直分布を それぞれ Text-fig. 5, 6 に図示した．

この観測は秋季の降温がかなり進行した時期に実施した．午前の観測は満潮時を中心に，午後の観測は下げ潮末期に行なった (Table 4, Text-fig. 2)．従って，潮時からいえば，定期観測 I より 1.5～2 時間早目に観測したわけである．天候は晴，午前は無風，午後は南寄りの微風，観測時の海上気温の平均は午前 19.0°, 午後 18.2° で表層水温の平均 17.8° より少し高い．

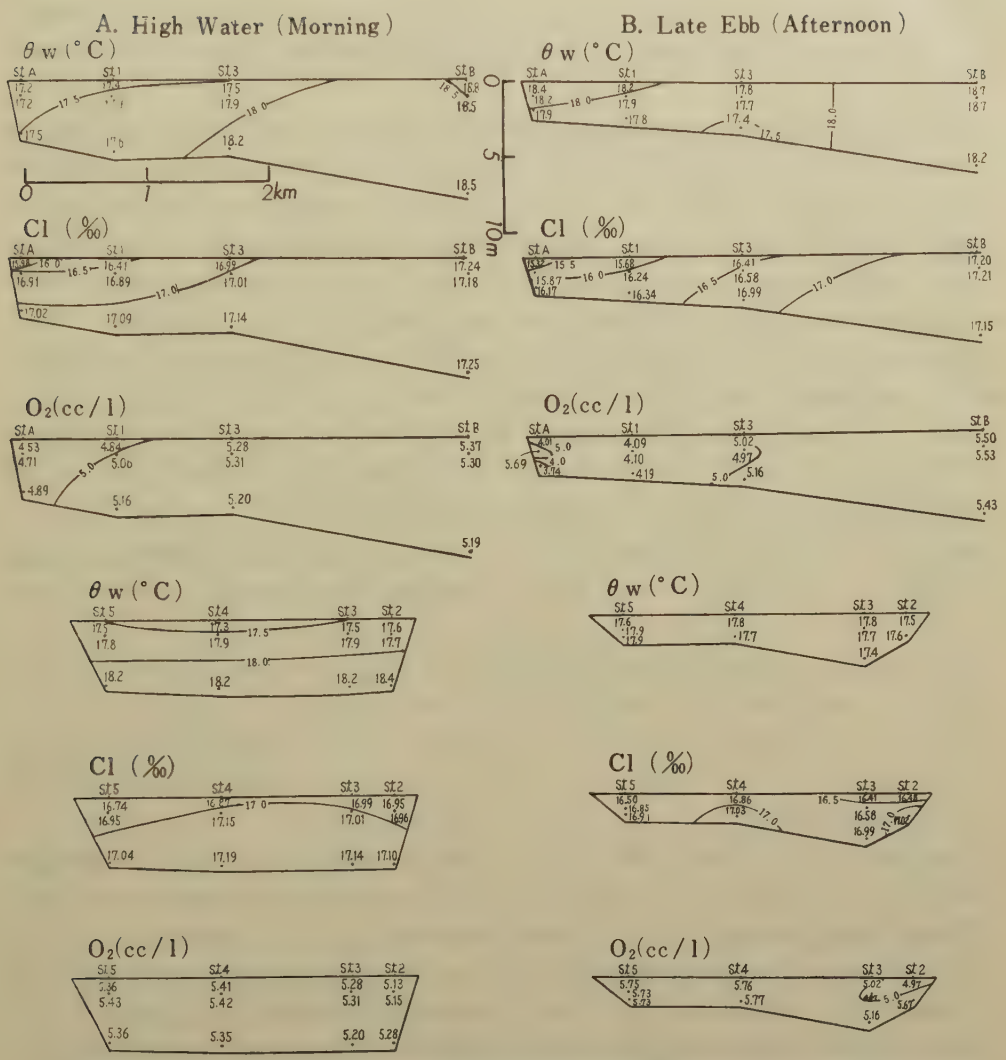
水　温 (Text-fig. 5A, B, 6)：　午前午後の各測点各深度を通じ大体 17.2～18.4° の範囲であったが，最も沖側の St. 8 の上層は午前も午後もこれより高かった (18.5～18.8°)．




Text-fig. 5. Horizontal distribution of water temperature ($\theta_w$), chlorinity (Cl), dissolved oxygen ($O_2$), chemical oxygen demand (C.O.D.) and transparency (Tr) in the two observations on November 10, 1958.

Light-faced letter and solid line····· High water in the morning
Thick letter and dotted line ····· Late ebb in the afternoon

表層—底層間の温度差は小さく，多くの場合0.4°以内であったが，午前の満潮時には一般に底層の方が高温であるのに対し，午後の下げ潮末期には表層の方が高温であった．ただし




Text-fig. 6. Vertical distribution of water temperature ($\theta_w$), chlorinity (Cl) and dissolved oxygen ($O_2$) in the two observations of November 10, 1958.

沖側の St. 8, 10, B では午前・午後とも表層の方が高温であった．

水温は一般に沖から陸岸へ向って低くなる傾向を示したが，下げ潮末期に陸岸にごく近接した測点の上層が逆に周囲より多少高温を示す場合があった（St. A, 1, 6 など）．

各測点各深度とも，午前・午後の観測の間に水温はわずかしか変化せず，変化は大体 ±0.3° の範囲であった．ただし，陸岸に近い測点の上層が午後（下げ潮末期）には午前（満潮時）より 0.5～1.0° 昇温している場合があった（St. A, 1, 4, 6 など）．

大学南岸に近い St 10 の表層水温は 18.1° で，Text-fig. 3 の季節変化と一致する．

塩素量（Text-fig. 5C, D, 6）：塩素量は沖側の St. 8, 10, B で最も高く，表層で 17.04～17.24‰を示した．これらの測点では表層－底層間の差は小さく（0.11‰以下），潮汐による変化もわずかであった（0.10‰以内）．

各深度の塩素量は，これらの測点から陸岸へ向って低下し，福山・釜屋両入江内の表層で最低を示した（16‰以下）．ただし満潮時の底層の塩素量は岸寄りの測点でも 17‰ 以上あった．

2 m 等深線以西の測点では，下げ潮末期には満潮時より一般に塩素量が低く，この低下は南西部（St. 4, 5）ではあまり目立たず，浚渫航路ぞいの St. A, 1, 3 に顕著であり，前者で約 0.1‰，後者で 0.4～1.0‰ 低下した．さきにのべたように，定期観測 I では これらの測点の塩素量は低潮時に表層だけが 1.0‰以上低下し，1m層はほとんど変化がなかった．それに比べると，今回は塩素量の変化は小さいが 1 m 層でも低下が起っており，また主として福山入江・浚渫航路付近に顕著な低下が見られた点が前回と異なっている．この原因については，各入江・樋門の放水量，樋門の開門時刻などの資料がないため定量的な考察はできないが，午前と午後の観測時の潮位の差が定期観測 I では 1.2mであったのに対し今回は 2.5mと約 2 倍であり（Text-fig. 2），また今回は陸水が海水より低温であったから，陸水・海水の混合は今回の方がすみやかに進行しえたであろうという点を指摘しておく．学部南岸付近の St. 10 の表層塩素量は 17.05‰程度で，さきに推定した季節変化（Text-fig. 3）よりかなり低い．

鉛直安定度： 鉛直安定度は各測点とも小さい．前述のように水温の鉛直傾度が潮時によって逆転したことを考えあわせると，各測点とも鉛直混合が盛んであったと見なしてよいようである．

E′ が最も小さいのは沖側の St. 8, 10, B で，各層とも潮時にかかわらず大体 0 ～0.06 の範囲であった．その他の測点では 1m以深は0＜E′＜0.1の場合が多く，0—1m層の E′ はこれよりやや大きく，また潮時により変化した．なお満潮時に St. E 表層の塩素量は14.84‰で著しく低かったが，このとき 0—1m間の E′ は -2.3 であったから，その後下方の海水と速かに混合したものと思われる．

溶存酸素量（Text-fig. 5E, F, 6）： 一般に，午前の満潮時には 5.0～5.5cc/L（飽和度88～97‰），午後の下げ潮末期には各点各層ともこれより0.2～0.4cc/L増加した．しかし，福山入江起源の貧酸素水の影響を受けた水層にはこの通則があてはまらず，酸素量は 5cc/L以下であり，下げ潮末期には満潮時より酸素量が低かった． このような水層は満潮時には St. A の全層および St. 1 の表層だけであったが，下げ潮末期には浚渫航路ぞいに拡がり，St. 3 の表層にまで及んだ．このほか，満潮時の St. E の表層・1m層も酸素量がやや低かった．

酸素量の鉛直傾度は，福山入江起源の貧酸素水の分布する水域では正（下層ほど酸素が多い），その他の水域では 一般に負であったが，いずれの場合も 表層—底層間の酸素量の差は 0.2cc/L 以内の場合が多かった．

C.O.D. (Text-fig. 5H, I)： 福山入江口（下げ潮末期の St. A, 1）および西岸寄りの St. C（満潮時の表層）などに見られた高値を除けば，測定値はすべて 1.1～1.8 ppm の範囲に入る．表層の C.O.D. は満潮時・下げ潮末期とも沖側の St. 8, B などで 最も小さく，陸岸へ向ってわずかに増大する傾向を示すが，底層ではこの傾向は明らかでない．各測点の各深度について，午前（満潮時）と午後（下げ潮末期）の値を比較すると，午後の方が高い場合が多く，その差は沖側の測点で 0.2 ppm 程度，福山入江口付近では 0.6 ppm 程度であった．鉛直分布は区々で，法則性を見出しがたい．

酸可溶性鉄： 測定値の 大部分は 0.05 ppm 以下であった．これより 大きい値は 陸水の混入度の高い水層または底層に見られ，下げ潮末期の福山入江口（St. A の全層，St. 1 の表

・底層）では特に大きく 0.21～0.28 ppm であった．これらの測点では満潮時には 0.15 ppm 以下であったから，下げ潮時に福山人江を流下して来た水に鉄が多く含まれていたと考えられる．

透明度（Text-fig. 5G）：　沖側の St. 8, 10, B で最も大きく満潮時に 3.0～3.5m を示す．満潮時の透明度はこれらの測点から陸岸へ向って次第に減少するが，最も陸寄りの測点でも 2.0m以上である（ただし福山入江内の St. A は 1.8m）．下げ潮末期には，福山入江の水の影響を受ける St. A, 1, 3 の透明度が 1.0～1.5m に減少し，同時に前記の沖側の 3 測点の透明度も 0.2～0.5m減少した．

水　　色：　満潮時には沖側の St. 8, 10, B で 6，表層が著しく低塩分であった St. 6, E で 8～9，その他の測点では 7 であった．下げ潮末期には，沖側の 3 測点の水色は高潮時よりわずかに悪化し（6, 6～7, 7），福山入江口の St. A, 1 では暗色が強くて測定できず，その他の測点では 7 であった．

総　　括：　以上の結果を総括すると，

① 秋の冷却期にあたり，鉛直安定度は 9 月の観測におけるよりもさらに小さい．水温・塩素量・溶存酸素量の鉛直傾度は一般に小さく，上・下層の混合がよく行なわれていたと推定される．水温・塩素量は沖側に高く，一般に陸岸に近ずくにつれて低下する．水温は既往の記録にもとずく平年値とほぼ等しいが，塩素量はこれよりかなり低い．

② 沖側（St. 8, 10, B）では水温が高く，塩素量・溶存酸素量・透明度が大きく，水色がよく，鉄量・C.O.D. が小さく，上・下層の混合が最も進んでおり，また潮時による水温・塩素量の変化が小さい．

③ 満潮時には塩素量の高い水が下層にさしこみ，陸岸にまで達する．

④ 福山入江内では，塩素量・溶存酸素量・透明度が小さく，鉄量・C. O. D. が大きく，水色が悪い．低潮時には浚渫航路ぞいの測点にこの水の影響が顕著に見られた．また釜屋入江付近からも低塩分の水が放出され，低潮時に 2 m等深線以西の各測点にその影響が認められたが，あまり顕著ではなかった．箕島東岸の St. 6 は透明度が小さく水色が悪く，表層の塩分が低く，芦田川川口起源の低塩分水が分布していたと考えられる．

⑤ 酸素量は，福山入江の水に影響されない水層では，午前の満潮時には一般に 5.0～5.5 cc/L（飽和度88～97%）で，午後には一般にこれより増加し過飽和に達した測点もあった．鉛直傾度は小さかった．

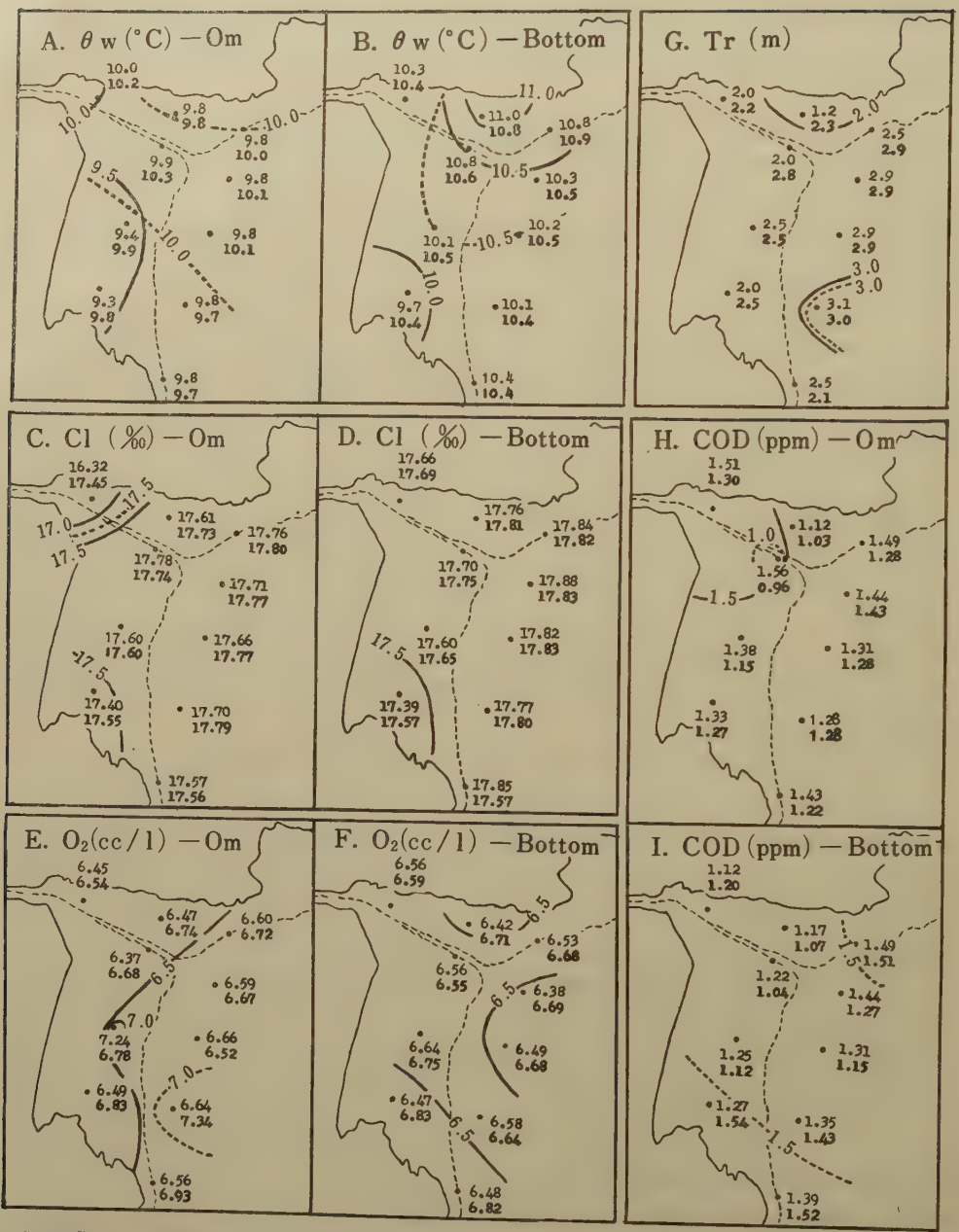
⑥ C. O. D. および酸可溶性鉄は陸水（ことに福山入江水）の混入により増大した．C. O. D. は午前（満潮時）より午後（下げ潮末期）の方が多少大きい傾向が見られた．また一般に底層は中層より鉄量が多かった．

(3)　定期観測 III（1959年3月13日）

観測データは Table C-1, C-2 の通りで，主要な要素の水平分布を Text-fig. 7 に示した．

この観測は春の昇温の始まった時期に実施した．他の定期観測と異なり，午前の観測は上げ潮中期に，午後の観測は下げ潮中期に行なった（Table 4, Text-fig. 2）．天候はくもり，観測時の海上気温の平均は午前 6.1°，午後 7.9°で，表層水温の平均よりそれぞれ約 4° および 2° 低かった．

学部南岸に近い St. 10 の表層は水温 9.9°，塩素量 17.8‰ で，Text-fig. 3 の季節変化と一致し，塩素量が最も高い季節にあたる．




Text-fig. 7. Horizontal distribution of water temperature ($\theta_w$), chlorinity (Cl), dissolved oxygen ($O_2$), chemical oxygen demand (C.O.D.) and transparency (Tr) in the two observations on March 13, 1959.

Light-faced letter and solid line ····· Flood in the morning
Thick letter and dotted line ····· Ebb in the afternoon

水温 (Text-fig. 7A, B)： 午前・午後を通じ 9.3～11.0°の範囲で，各測点とも表層から下方へ向ってわずかに高くなり，表・底層間の差は 0.3～1.2° であった．水温が最も高かっ

たのは北東部（St. 2, 3, 10 など）の底層で，これに対し釜屋入江寄り（St. 5 など）は比較的低温であった．各点各層の水温は午前・午後相等しいか，または午後の方が少し（0.5°以下）高かった．

塩素量（Text-fig. 7C, D）：　全般的に塩素量が高く，少数の例外を除き各点各層とも17.5‰以上であった．一般に 0－1m層間は塩素量はほとんど差がなく，1m以深は差がないかまたは下方へ向ってわずかに増加した（1m—底層間の差は 0.3‰以内）．塩素量が最も高いのは沖側北部（St. 8～10）の底層で，上げ潮・下げ潮時とも 17.82～17.88‰で，St. 2（下げ潮時）・St. 6（上げ潮時）の底層にもこの程度の高い値が見られた．塩素量が低かったのは福山入江・釜屋入江付近で，最低は16.22‰（St. 1 表層，上げ潮時）であった．各点各層の上げ潮・下げ潮時の塩素量を比較すると，相等しいかまたは下げ潮時の方が少し高く，差は一般に0.2‰以内であった．

鉛直安定度：　0—1m層間は午前・午後ともに負で（$-1.6 \leq E' \leq 0$），これは気温が低く表層が1m層より低温であったことによる．1m－底層間も鉛直安定度が小さく，E′の値は午前は 0～0.1．午後は一般に 0 であった．従って，昇温期ではあるが鉛直成層は発達していなかったといえる．

溶存酸素量（Text-fig. 7E, F）：　測定値は少数の例外を除き 6.4～7.0cc/L（飽和度 97～105%）の範囲で，一般に表層から底層へ向ってわずかに減少するが，底層でも飽和度 98%以上である．同一測点の同じ層の午前と午後の値を比較すると，多くの場合午後（上げ潮時）の方が 0.1～0.3cc/L 程度大きい．福山入江口付近（上げ潮時の St. 1～3，下げ潮時の St. 1）の表層は周囲より酸素量がやや小さく，これは入江の水の混入によると思われるが，周囲との差は前2回の観測の場合よりはるかに小さい．なお，表層または 1 m層の酸素量が周囲から孤立して高い測点があった（St. 4, 7, 10）．

C. O. D.（Text-fig. 7H, I）：　全般的に 1.0～1.6 ppm の範囲で，各点各層とも午前・午後の値の差はわずかである．午前（上げ潮時）には St. 1, 3 の表層および St. 10（全層）などが，午後（下げ潮時）には St. 5, 6, 10 の底層が，周囲よりやや大きい C. O. D. を示した．他の観測の場合とことなり，福山・釜屋入江付近が特に高い値を示すということはなかった．

酸可溶性鉄：　一般に 0.05 ppm 以下であった．これより大きい値は，主として上げ潮時に福山・釜屋入江付近の塩分の比較的低い水層に見られた（St. 1, 2, 5）．このほか St. 5, 6, 10 などの底層がやや高い値を示した．

透明度（Text-fig. 7G）：　上げ潮・下げ潮時を通じ最も陸寄りの測点でも 2.0m以上あり（St. 2 上げ潮時の 1.2mは例外），最も大きい値は沖側の St. 7～9 に見られた（2.9m以上）．浚渫航路下流部の St. 3 では下げ潮時の透明度が沖側とほとんど等しく，沖側の水の影響が強く及んでいたと推定される．

総　　括：　以上を総括すると，

①　塩素量が最も高い季節にあたる．春の昇温が始まっているが鉛直成層は発達しておらず，鉛直安定度は全層を通じ 0 に近い．水温・塩素量の鉛直分布は，表層—1 m層間は塩素量はほぼ等しく水温は表層が低い．1 m以深は水温・塩素量とも下層へ向ってわずかに増大する．

②　水温・塩素量・透明度の最も高い水は沖側北寄り（St. 8～10）に分布するが，浚渫航路ぞいの St. 2, 3 などでこれに劣らぬ高い塩素量または透明度が見られた．

③ 福山・釜屋入江付近における流入陸水の影響（塩素量・酸素量・透明度の減少，C. O. D., 鉄量の増加）は他の観測の場合よりはるかに軽微で影響範囲も局限されていた．換言すれば，沖側の水の影響が水域全体に強く及んでいた．これは，干潮時に観測を行なわなかったことによる見かけの傾向ではなく，季節的な傾向であろうと考えられる．

④ 上げ潮時には下げ潮時に比べて，両入江付近における陸水の影響範囲がやや広く，他の測点においても塩素量・透明度の低い場合が多かった．

⑤ 溶存酸素はどの測点でも底層まで飽和に近く，同じ測点の同じ深度の午前・午後を比較すると，一般に午後（下げ潮時）の方が 0.1～0.3cc/L 程度多かった．

(4) 定期観測 IV（1959年7月1日）

観測データを Table D-1, D-2 に，おもな要素の水平・鉛直分布をそれぞれ Text-fig. 8, 9 に示した．

この観測は水温が年の最高に近ずいた時期に実施したもので，午前の観測は下げ潮の中期に，午後の観測は干潮直後（上げ潮の初期）に行なった（Table 4, Text-fig. 2）．天候はくもり，観測点における気温の平均は午前 25.3°，午後 27.1° で，表層水温の平均よりそれぞれ 1°, 0.1° だけ高い．学部南岸に近い St. 10 の表層水温は 26.8°（午前・午後の平均）で沿岸水温の平年値に近く，表層塩素量は 17.42‰（同上）で比較的低く，この時季が塩分の年変化の一つの谷にあたっていることと一致する（Text-fig. 3）．

水 温（Text-fig. 8A, B, 9）： 沖測中央部（St. 7, 8, B など）の底層が最も低温で，最低は 24.0° であった（St. 7, 下げ潮時）．ここから表層および陸岸へ向って水温が高くなり，最高は午後（上げ潮初期）に福山入江口の表層に見られた 28.9° である．

沖側の測点では午前・午後の水温に著しい差がなかったのに対し，2 m 等深線以西の測点では，午後の上げ潮初期には午前にくらべて，各層とも 0.5～2.0° 高温であった．このため，午後の各層の水温は 2m等深線の岸側と沖側とでは明瞭な差を生じた．

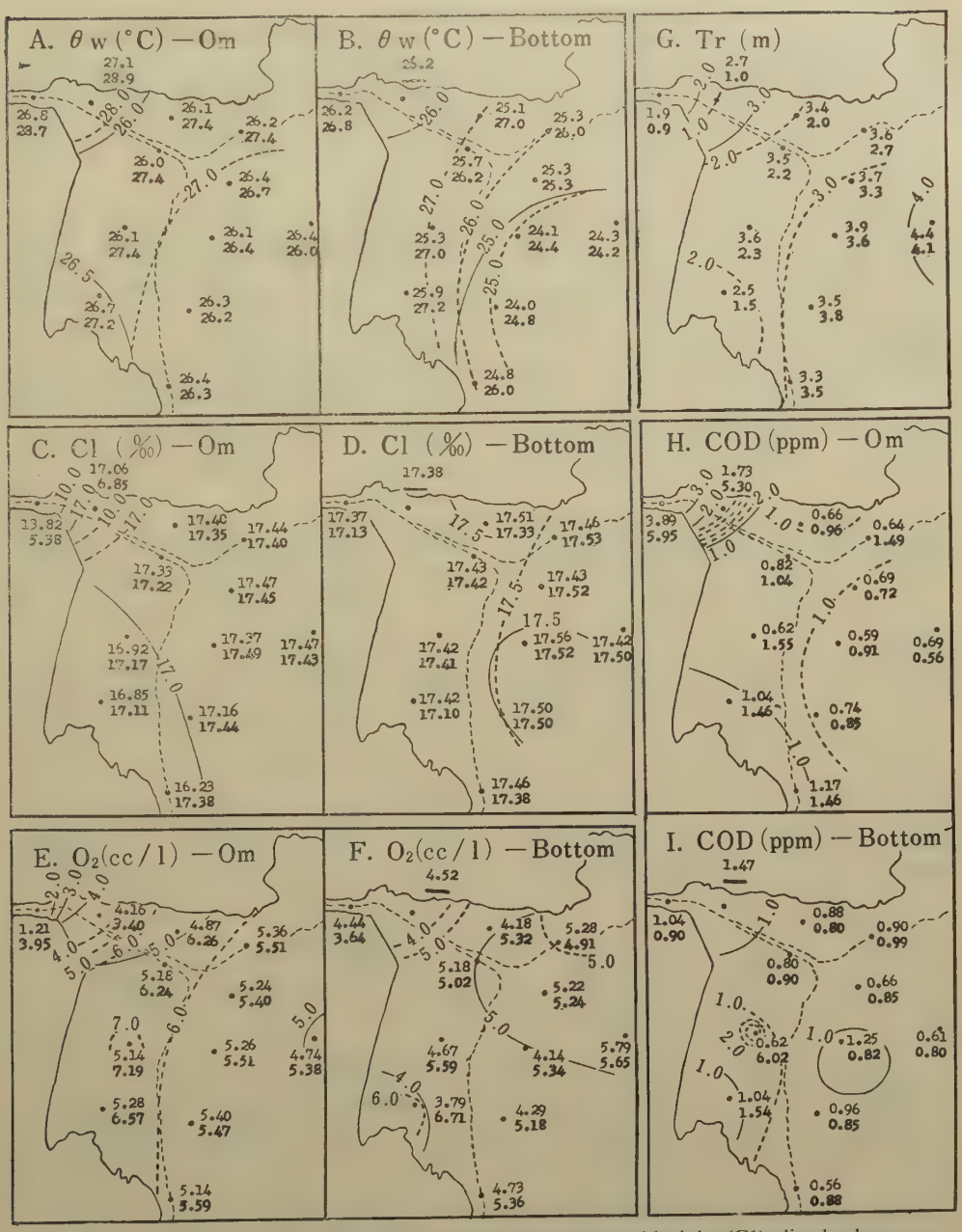
塩素量（Text-fig. 8C, D, 9）： 午前・午後を通じて沖側中央部（St. 7～9, B など）の底層が最も高く，最高は 17.57‰であった（St. 8, 上げ潮初期）．ここから表層および陸岸へ向って塩素量は徐々に低下するが，最も岸寄りの測点でも底層は一般に 17.3‰ 以上であった．一方，陸水の混入したやや低塩分（Cl<17.2‰）の水が，福山入江口では局所的に（St. A, 1），南西部ではやや広範囲に（St. 4～6 など），上記の底層水の上に張り出す．従って，高塩分の水の勢力は沖側中央部から福山入江口の方向にむかっていたとの印象を受ける．

福山入江口における塩素量の低下は干潮時に著しく，表層は 5.4～6.9‰ となり，入江に通ずる浚渫航路にある St. 3 の表層も 17.22‰ に低下した．これに対し南西部の低塩分域の塩素量は，下げ潮時の方が干潮時より 0.2‰ 程度低かった．

鉛直安定度： 一般に $0 < E' < 0.3$ であったが，表層が低塩分を示した St. A（両潮時）と St. 6（下げ潮時）の 0—1m層間では 1.8～12 という大きな値が見られた．また上げ潮初期には南西部の St. 4～6 および他の 2～3 の測点の 0—1m層間の $E'$ が負（0～-0.2）であった．

溶存酸素量（Text-fig. 8E, F, 9）： 午前・午後ともに一般に酸素量は豊富で，測定値の大部分は 4.4～7.2cc/L（飽和度90～150%）の範囲で，これより小さい値を示したのは，福山入江水の影響が顕著な水域（St. A, 1）および下げ潮時の St. 2, 5, 7, 8 の底層である．

酸素量の鉛直傾度は測点によりまちまちであるが，福山入江水の影響を受けた水域を除け

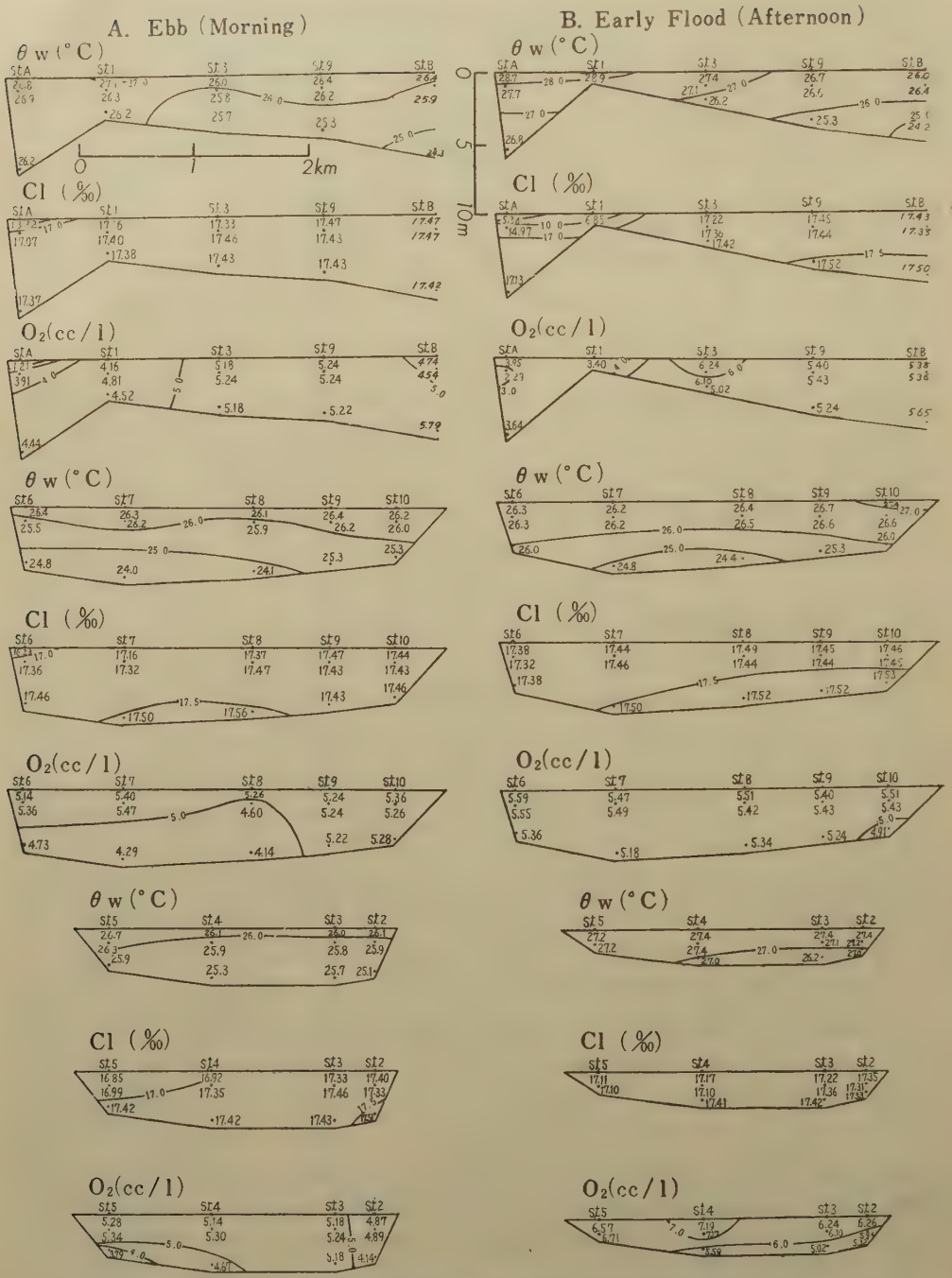



Text-fig. 8. Horizontal distribution of water temperature ($\theta_w$), chlorinity (Cl), dissolved oxygen ($O_2$), chemical oxygen demand (C.O.D.) and transparency (Tr) in the two observations on July 1, 1959.

Light-faced letter and solid line····· Ebb in the morning

Thick letter and dotted line ····· Early flood in the afternoon

ば，表層から底層までほとんど変化がないか，または表層—1 m層間ではあまり差がなく底層がこれよりかなり少ない場合が多かった．




Text-fig. 9. Vertical distribution of water temperature ($\theta_w$), chlorinity (Cl) and dissolved oxygen ($O_2$) in the two observations of July 1, 1959.

底層の酸素量は，午前の下げ潮時には上記のようにやや少ない場合もあったが，午後の上げ潮初期には全点過飽和を示した．なお，最も沖側の St. B では午前も午後も底層の方が

0, 1m層より酸素量が大きかったが，その理由は明らかでない．

沖側の各測点の0, 1m層の酸素量は午前と午後とであまり差がないが，2m等深線以西の測点（St. 2～5）では，0, 1m層の酸素量は午後には午前より1～2cc/L増加した．その結果，午後の0, 1m層の酸素量は，水温の場合と同じく，2m等深線付近を境にして明瞭な差を示した．

C. O. D.（Text-fig. 8H, I）：　0. 5～0. 9 ppmの場合が多く，他の定期観測の値よりやや小さいように思われる．沖側の測点（St. 7～9, Bなど）の各層は午前・午後とも大体この範囲にあり，岸寄りの測点も午前の下げ潮時には，福山入江口および南西部を除き，この範囲にあった．午後の上げ潮初期には，福山入江口表層のC. O. D.は著しく増加して5. 3～5. 95 ppmとなり，2m等深線以西の他の測点の0, 1m層でも一般に増加して0. 9～1. 6 ppmとなり沖側よりかなり高くなっていた．

酸可溶性鉄：　測定値は大部分が0. 03 ppm以下であったが，福山入江口のSt. Aの各層とSt. 1の表層は両潮時に，多くの測点の底層とSt. 5, 6の表層は下げ潮時に，これより大きい値を示した．

透明度（Text-fig. 8G）：　沖側中央部のSt. 7, 8, Bでは両潮時を通じて3. 5m以上で最も大きく，ここから陸岸へ向って減少し，福山入江・釜屋入江付近が最も小さい．一般に下げ潮時より干潮時の方が透明度が小さく，この傾向は2m等深線以西で特に顕著であった．

水　　色：　沖側の測点（St. 7～10, B）では一般に5～6で潮時による差は小さい．2m等深線以西の測点ではこれより水色が悪く，下げ潮時には6～7，上げ潮時には7以下の場合が多く，福山入江内（St. A）および釜屋入江前（St. 6）は最も水色が悪かった．

総　　括：　以上の観測結果は次のように総括できよう．

①　水温は年の最高に近く，塩分は比較的低い．表層水温は気温より少し低い．鉛直成層の発達は著しくなく一般に$0<E'<0.3$であるが，陸水流入口付近の0—1m層間のE′はこれより大きい．また上げ潮初期に0—1m層のE′が負となる測点があった．水温は表層から底層へ向って低下するが，表・底層間の差は一般に2°以内である．塩素量は，陸水の混入が顕著な場合を除けば，鉛直方向の変化は小さい．

②　沖側中央部（St. 7, 8, Bなど）には陸水の影響の最も少ない水が分布したと考えられ，透明度高く水色よく，水温低く塩素量高く，C. O. D.および鉄量が小さい．ここでは各要素の潮時による変化が小さく，底層は全水域中で最も低温・高塩分である．

③　陸水の混入した低塩分の水は福山入江口と南西部のそれぞれ上層に分布し，前者では分布範囲は比較的局限され後者ではやや広範囲に分布したと考えられる．これらの低塩分水の分布する水域（ないし水層）は，透明度が小さく水色が悪く，高温でC. O. D.および鉄量の大きいのが特長であったが，福山入江口の場合には溶存酸素の減少も顕著であった．なお，箕島東岸のSt. 6に見られた低塩分水は，潮汐にともなう動向から見てSt. 4, 5の低塩分水とは連続せず，恐らく芦田川口起源のものであろうと想像される．

④　沖側の高塩分水はこれらの低塩分水の下方に拡がり，その影響は浚渫航路に向って強かった．

⑤　溶存酸素は豊富で，福山入江水の混入しない水層では一般に飽和度90%以上であった．底層の酸素量は下げ潮時にはやや少ない場合があったが，午後の上げ潮初期には全点過飽和を示した．2m等深線以西の浅所では，0, 1m層の酸素量が昼間の干潮時に著しく増加し，

同時に各層の水温がかなり上昇した.

⑥ C. O. D. は 0.5～0.9 ppm の場合が多く，他の定期観測の値より多少小さいように思われた.

(5) 12時間観測（1960年7月10日）

この観測は潮汐にともなう福山入江口付近の水の流動・交代を調査したもので，福山市引野漁協前の浚渫航路に観測艇を碇置して行なった（Text-fig. 1 の St. T）. 観測項目・観測層は Table E の通りで，測定は 5時30分から17時30分まで 1時間おきに行ない，表層では毎回採水したが，1, 2m 層は水深の増減に応じどちらか一方で採水した. Table E に示した値のほかに，サーミスター水温計で表層から底層までの水温を 1m おきに測定し，また干潮・満潮時前後には数分おきに水深を測った. 当日の日出・日入時刻，潮時などを Table 5 に示した. 観測結果のうち，おもなものを Text-fig. 10 に図示した.

Table 5. Sunrise, sunset and tide of July 10, 1960

| | Sunrise | Sunset | Low Water I | | High Water I | | Low Water II | | Rise of tide level from Low Water I to High Water I |
|---|---|---|---|---|---|---|---|---|---|
| | | | Time | Tide level | Time | Tide level | Time | Tide level | |
| Predicted | ♯ $4^h58^m$ | ♯ $19^h20^m$ | ※ $5^h53^m$ | ※ 1.1m | ※$11^h32^m$ | ※ 3.5m | ※$17^h54^m$ | ※ 0.1m | ※ 2.4m |
| observed at St. T | — | — | 6 30 | — | 11 40 | — | 18 30 | — | 2.6 |

♯ Predicted time for Okayama[33] (34° 40′ N, 133° 56′ E).

※ Predicted time or value for Minoshima.

天候は始めはくもり，その後雨，晴と変り，これにともない風向・気温も変化した. 気温は常に水温より高かった. 観測日に先立ちかなり大量の降雨* があったため，観測点の北西約 800m にある農地排水樋門から干潮時を中心に大量の淡水が放出され，その影響が観測点に及んだ.

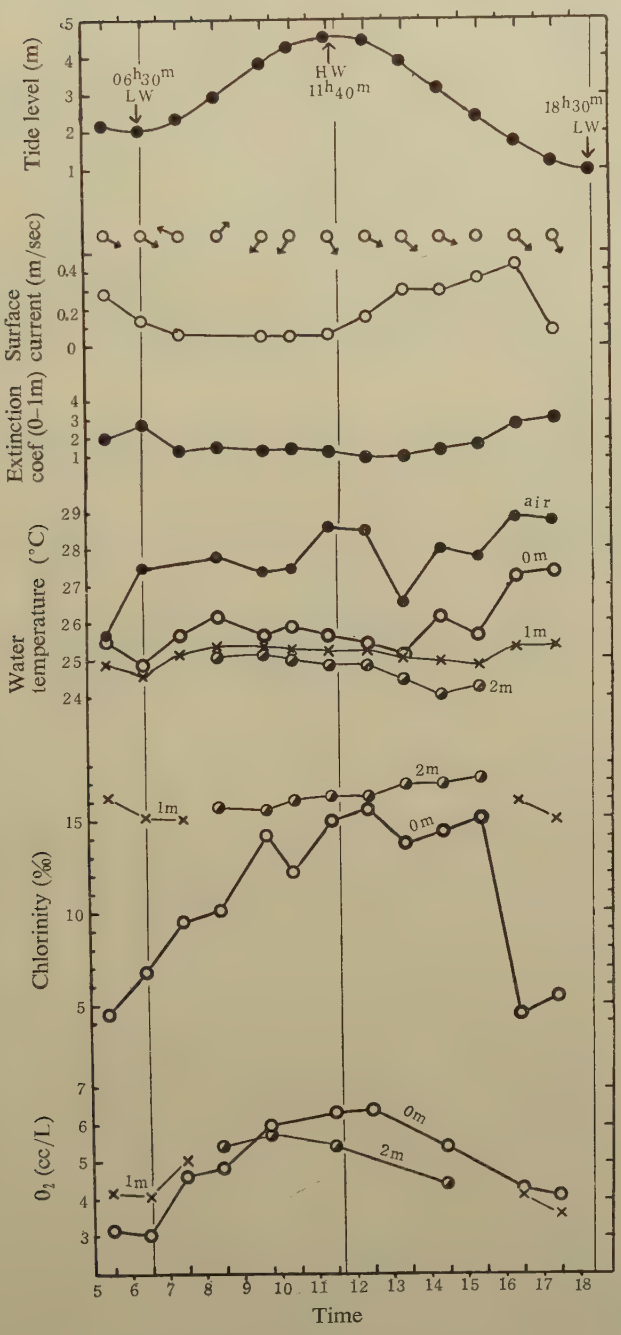
観測点の水深は満潮時に 4.6m，午後の下げ潮末期に最低（1.3m）となった. 水深の時間的変化から推定した現場の潮汐は，箕島の推算値にくらべて，満潮は 8分，干潮は 36～37分遅れ，午前の干・満潮間の潮差は 0.2m だけ大きかった(Table 5). これらは地形の影響——観測点が長い入江に通ずる水路にあったという事情——によるものであろう.

表層潮流は，下げ潮時には流向 SSE ～ ESE でほぼ水路に沿って流下し，流速は 7～45 cm/sec でかなり速い. 樋門から放出された淡水がこの下げ潮流にのって流下した. 一方，上げ潮時には，水路をさかのぼる方向（WNW）またはこれと直角方向（NE, SW など）に流れ，流速は 6～7cm/sec で小さく，水が水路をさかのぼりつつ周囲へあふれ出しているかのような印象を受けた.

透明度は，満潮時前後には 1.5～1.9m，干潮時前後には 0.5～0.8m であった. 干潮時に透明度が小さいのは，樋門から放出された陸水および福山入江の水が混入したためで，低塩

* 松永測候所（学部東方約 16km）における降雨量は次の通りであった.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7月 | 1～5日 | 29mm | 7日 | 78.2mm | 9日 | — | 11日 | — |
| | 6日 | 0.1mm | 8日 | 84.2mm | 10日 | 5.1mm | | |




Text-fig. 10. Results of the 12-hour observation at St. T on July 10, 1960.

分のときに透明度が小さい．0 -1m層の消散係数* も透明度と同様に，干潮時に水が濁っていたことを示し，その値は満潮時に小さく（1.0～1.4），干潮時に大きく（1.9～3.1），表層の塩素量を明瞭な逆相関を示す．

水温は表層が最も高く，下層へ向って低くなる．表層水温は24.9～27.4°の範囲で変化し，早朝の干潮時に最も低く夕方の干潮時に最高となったが，この間単調に昇温したのではなく日中に降温の続く場合があった．これは潮汐による水塊の交代のほか，水深・気温・日射などの変化によると考えられる．1m層の水温は24.6～25.4°で変化の巾は小さいが，その昇降は表層水温に似た傾向を示した．

塩素量は，1, 2m層では15～17.3‰の範囲で変化は比較的小さい．表層では変化が大きく，下げ潮末期には主として樋門から流出した陸水のために5‰前後に低下して最低となり，その後上げ潮時に次第に上昇し，満潮時ないしその直後に最高（15～15.5‰）に達した．

pHは一般に8.1であったが，下げ潮末期・干潮時の表層は陸水・入江水の混入にともない7.2またはそれ以下となった．

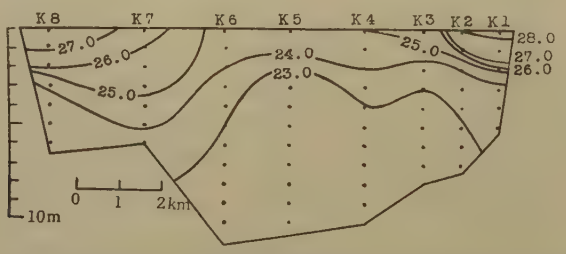
溶存酸素量は，表層では潮位の昇降にともなって増減し，昼間の満潮時前後に最も高く6.4cc/l（飽和度135%），干潮時前後に最も低く3.0～4.3cc/l（53～78%）であった．潮位の降下にともない酸素量が減少したのは，主として福山入江内の貧酸素の水が流下して来たためであろう．1m層の酸素量は早朝の干潮時には表層よりかなり大きいが，夕方の干潮時には表層よりわずかに小さかった．2m層の酸素量は一般に表層よりも小さい値であった．

以上を要約すると，今回の観測中最も顕著な現象は，農地排水樋門から放出される陸水のために，下げ潮末期・干潮時を中心に表層の塩分が低下し消散係数が増大したことである．このほか潮位の昇降にともなって透明度・溶存酸素量がそれぞれ増減したが，これは樋門の放出水・福山入江水の混入によるものと考えられた．

(6) 流入陸水調査（1960年7月11日）

この調査は，7月7, 8日の豪雨**による流入陸水の影響をみるために，笠岡湾中央部において3線8点の横断観測を行ったものである（Text-fig. 1の測点K 1～8）．観測項目は，サーミスター水温計による1m層ごとの水温，表層および下層の塩素量，および透明度で，観測はほぼ満潮時に行なった．

観測データをTable Fに，水温の鉛直分布をText-fig. 11に示した．




Text-fig. 11. Vertical distribution of water temperature (°C) in the section passing St. K1～K8 on July 11, 1960.

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* 水中照度計（セレニウム光電池．白色拡散板および視感度フィルター付）を用いて水面直下と1m層の照度を測定し，これより計算した値．

** 266頁の脚註参照．

水温は，表層では笠岡湾口の中央から東にかけての測点 K4～K6 で最も低く 24.3～25.0 °C であった．これらの測点を中心に湾奥すなわち陸岸に近ずくに従って高くなり．笠岡側の奥部 K1 では 28.1°C，福山側の奥部 K8 では 27.5°C となっていた．中・底層の水温は，これらより低く，特に 7m以上の水深をもつ測点 K2～K6 では 5m以深の水温は 22.7～23.0 °C でほぼ一様である．

塩素量の分布は水温のそれとよく一致しており，表層では湾口の中央から東にかけての測点 K3～K6 で 16.6～17.0‰と高く，湾奥へ進むに従って低くなり，笠岡側の K1 では 13.18 ‰で最も低い．中・底層は表層に比べて塩素量が高く，特に 5m 以深は 17.32～17.48‰ で変化が少なく，水温の分布と考えあわせると 5m 以深はかなり均質であったと考えられる．

透明度は笠岡湾の常として一般に小さいが，湾口中央から東にかけての K3～K6 で 2.1～3.0m であるのに比べて，湾奥の K1, 2, 7, 8 では 1.3～1.9m で著しく小さく，水がにごっていたことを裏書きする．

以上の結果から見て，流入陸水の影響を強く受けたとみられる，透明度の小さい，高水温，低塩分の水の分布範囲は比較的狭く，鉛直方向には K7 を除けば 2m層までである．K7 では上記の低塩分水が 4m 層にまで及んだが，これは放水量の多い芦田川に近接しているためと考えられる．また水平的には K1, 2, 7, 8 に分布するにすぎず，距岸約 2km以内である．

笠岡湾においては潮汐にともなう海水の出入交代が大きいが，これにともなって流入陸水と海水の混合が促進され，その結果前者の分布範囲が見かけ上狭くなるものと考えられる．

なおこの調査は，満潮時に行なったものであり，干潮時には，流入陸水の影響範囲は表層ではさらに拡がるものと考えられる．

(7)　海況要素の変動について

以上の観測結果を通覧して，各観測要素の地域・季節・潮時による変化を要約すると次の通りである．

水　　温：　表層水温は 1～2月に最低（月平均 6～7°），8月に最高（同 28°位）に達し，年較差は21°に及ぶ．水温・気温の差は周年小さく，村上[20]（原著 p. 22）によれば，1952–53 年笠岡における水温・気温の旬平均の差は周年 2° 以下である．水平・鉛直分布については，夏（7月の観測IV）には底層よりも表層，また沖合よりも沿岸の方が水温が高く，他の季節（9, 11, 3月の観測 I, II, III）には逆に底層・沖合の方が高温である．

塩　素　量：　陸水流入口付近（St. 1, 5, A, D など）を除けば周年ほぼ 16～18‰ の範囲にあって変化が小さく，周年変化の谷は降水量の多い 7, 9月などに起る．水平・鉛直分布は，沿岸・表層に低く沖合・底層に高い傾向を示す．陸水流入口付近では，干潮時や大雨の後などに表層の塩素量が著しく低下することがあり，観測された最低値は 4.51‰（Table E）である．しかし，流入陸水の影響が 2m層にまで及ぶことはまれで，水平的にも影響範囲が局限されている．

溶存酸素量：　陸水流入口（特に福山入江口）ではしばしば低い値が見られるが，その他の測点では表層から底層（海底上0.3～1.5m）まで周年飽和に近いか過飽和である．鉛直的には，一般に表層より底層の方が酸素量が小さい．St. 4, 5 では昼間の低潮時に溶存酸素量が著しく増加する場合がある．その原因は充分には追究していないが，恐らく濃密な植物プランクトン群の光合成作用によるものであろう．

C. O. D.：　陸水流入口付近では 6 ppm に達する場合があるが，他の水域では 0.6～1.9

ppm である．周年変化は明瞭でない．

酸可溶性全鉄量： 一般に 0.05 ppm 以下であるが，陸水の影響の強い水および底層水ではこれより多く，測定した最大値は 0.64 ppm (Table D-1) である．山本ら[34]は海水の濁りと鉄量の間に密接な関係があるとしているが，これは本水域にもあてはまるように思われる．

pH ： 一般に 8.0～8.3 で 8.2 の場合が多いが，福山入江水が混入すると 7.2～7.5 になる場合がある．周年変化は顕著でない．

水色・透明度： 水色は，岸寄りでは悪く 7～9 で，沖側では 5～6 である．透明度は 1～4m の範囲で，岸寄りでは小さく沖側では大きい．水色も透明度も周年変化は明瞭でない．

潮　　流： 定期観測 I と 12時間観測のさいに潮流板を用いて表層の潮流を測定したのみで測定例が少なく，また下層の潮流は測定していない．

定期観測 I の上げ潮前期には，潮時の関係もあって流速は小さく (4～12cm/sec)，流向は岸寄りの St. 2, 4, 5 では岸に向かい，沖側の St. 7～10 では N～NE, 浚渫航路上の St. 3 では E であった (Table A-2)．下げ潮初期には，岸寄りの St. 1, 2, 4～6, 10 では流速 5～19cm/sec で沖へ向って流れるが，浚渫航路上の St. 3 では流向は NW であった (Table A-1)．

St. T における 12時間観測においては，さきにのべたように，上げ潮流は浚渫航路をさかのぼる方向に，下げ潮流は航路を流下する方向に流れ，流速はそれぞれ 6～7cm/sec, 16～45 cm/sec であった (p. 266)．この測点は浚渫航路のそばにあり，調査水域内では潮流が比較的速い地点に考えられるのに，流速はせいぜい上記の値であった．従って，調査水域内の潮流は一般にゆるやかであると考えられる．

本水域は，潮差が中等程度の場合，満潮時と干潮時の水量の比がおよそ 2:1 で，満潮時に水域内にあった海水の約半分が下げ潮時に水域外へ流れ去る計算となり，水の出入率が高い．それにもかかわらず潮流が上記のようにゆるやかなのは，本水域の位置が笠岡湾の潮流の主軸からはずれており (p. 277)，その形状が間口が広く奥行が小さく海底が平坦であるためと考えられる．

## 2. 底　　質

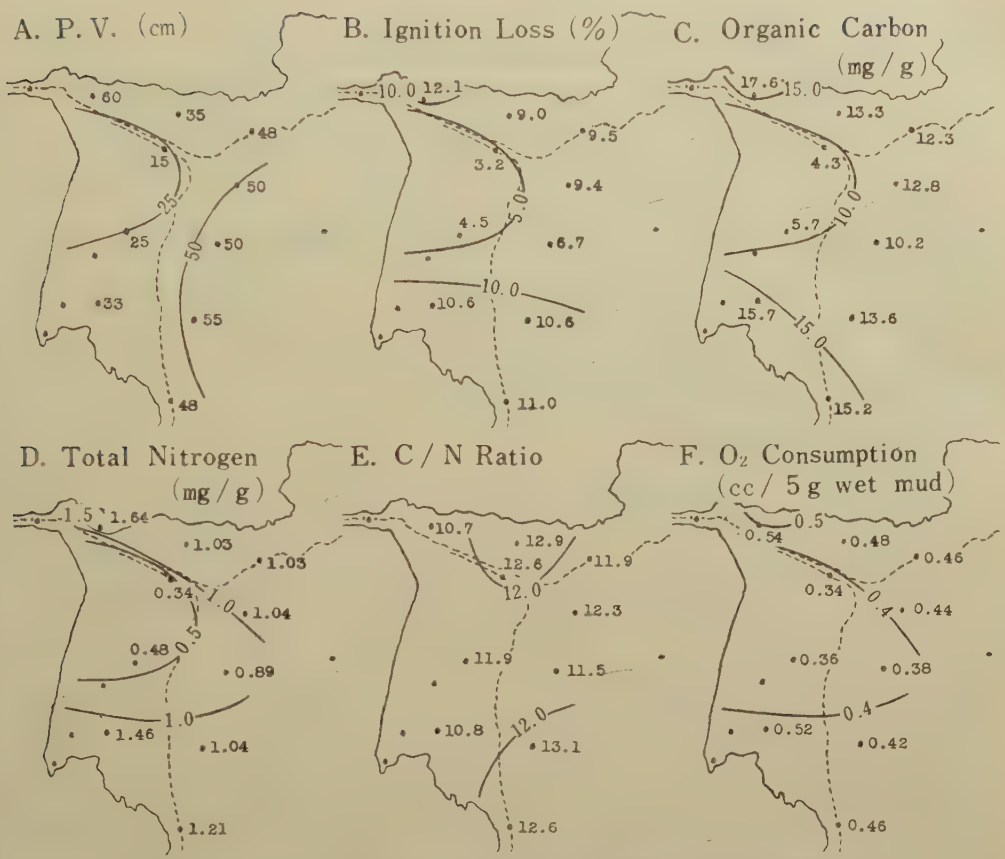
調査は 6 回行なったが，先ず第 5 回調査の結果にもとずいて各要素の地理的分布を説明し，その後各要素の変動と相互関係を考察する．

(1) 底質調査 V (1959年3月13日) における各要素の地理的分布

貫入深度 (P.V.) (Text-fig. 12A)： 浚渫航路付近にある St. 3 が最小値 (15cm) を示し底質が最も硬い．ついで 2m 等深線の陸側 (St. 2, 4, 5) は 25～35cm, 2m等深線の沖側 (St. 6～10) は 48～55cmと順に大きくなり，福山入江口の St. 1 が最大値 (60cm) を示す．一般に，底質が沈泥・粘土に富む場合に貫入深度が大きく，そのような底質は有機質に富む．

灼熱減量 (Text-fig. 12B)： 浚渫航路付近の St. 3 で最も小さく (3.2%)，その周囲の測点 St. 2, 4, 8～10 では 4.5～9.5%，西南部の St. 5～7 では 10.6～11.0%と順次大きくなり，福山入江口の St. 1 が最大値 (12.1%) を示した．

有機炭素量 (Text-fig. 12C)： 地理的分布は灼熱減量のそれとよく一致する．すなわち，浚渫航路付近の St. 3 が最小値 4.3mg/g を示し，その南側の St. 4 で 5.7mg/g，これらの沖側および北側の St. 2, 7～10 で 10.2～13.6mg/g，西南部の西南部の St. 5, 6 で 15.2～15.7 mg/g と順次増加し，最大値は福山入江口にある St. 1 の 17.6mg/g である．




Text-fig. 12. Properties of the bottom mud at regular stations on March 13, 1959.

窒素量 (Text-fig. 12D)： 窒素量の分布は 灼熱減量・有機炭素量の分布と 一致する．すなわち，St. 3 で最も小さく 0.34mg/g, その南側の St. 4 で 0.48mg/g, これらの沖側・北側の St. 2, 7～10 で 0.89～1.04mg/g, 西南部の St. 5, 6 で 1.21～1.46mg/g と順次増加し，最大値は St. 1 の 1.64mg/g である．

炭素率 (C/N) (Text-fig. 12E)： 10.7～13.1 で各測点とも似た値であるが，福山入江口の St. 1 および釜屋入江前の St. 5 の値がやや小さい．

底質の酸素消費量 (Text-fig. 12F)： 分布は 灼熱減量・有機炭素量・窒素量の それと同じ傾向を示す．浚渫航路付近の St. 3 では 0.34cc (湿泥 5 g 当り)，その南側の St. 4 では 0.36cc で共に小さく，これらの沖側・北側の St. 2, 6～10 では 0.38～0.48cc でやや大きく，入江口にあたる St. 1, 5 では 0.54, 0.52cc で最も大きい．

以上を通覧すると，福山入江口の St. 1, 釜屋入江口の St. 5 および芦田川口に近い St. 6 では，他の測点にくらべて，貫入深度が大きく (底質が軟かく)，灼熱減量・有機炭素量・窒素量・酸素消費量が大きく，炭素率が小さい傾向がみられる．これは，これらの測点が陸水の流入口に近く，底質が流入陸水の影響を強くうけることに起因すると考えられる．一方，浚渫航路付近にある St. 3 では，底質が 比較的硬く，有機質含量の指標となる 灼熱減量などの値が小さく，酸素消費量も小さい．これは，浚渫航路ぞいに流れる強い潮流によって軟泥が

洗い流されることに起因すると考えられる.

他の5回（窒素量の場合は1回）の調査結果をこれと比較すると（Table 6），同一測点における測定値が毎回少しずつ異なるが，地域による値の大小は上にのべた傾向とほぼ一致し，St. A, 1, 6, 7 では貫入深度および有機質含量の指標となる諸要素の値が大きく，St. 3, 4, 8 ではこれらの値が小さい．前者は流入陸水の影響，後者は潮流が比較的強く微細な粒子の沈積が妨げられるためとみなしてよいであろう.

各測点で測定値が毎回変化した原因としては，調査のたびに測点の位置が少しずつずれたことが考えられる．St. 1 では測定値の変動が比較的大きいが，この付近は底質が一様でないから測点の位置がわずかに移動しても測定値が大きく変化したものと考えられる．なお，各測点で酸素消費量の値がかなり著しく変動するのは，測点の位置がずれたことよりも，測定条件（実験温度）が毎回異なったことが主な原因と考えられる.

(2) 各要素の変動と相互関係

貫入深度（P.V.）： 6回の調査で測定した値は4～63cmの範囲にある（Table 6）．2m等深線以浅の測点では大体30cm前後であるのに対し，以深の測点では50cm前後で，2m等深線を境にして明らかな差がみられる．浅所を満潮時に，深所を干潮時に調査してもこの差が認められるから，この差は測器の落下距離によるものではなく，2m等深線の沖側の方が岸側より底質がやわらかいことを示していると考えられる．古川[4]が燧灘（水深14～31m）で測定した値は34～79cmで，本水域の値よりやや大きい.

灼熱減量： 6回の調査で測定された値は3.2～13.9%の範囲にあり（Table 6），加藤[13]が北海道北西域で得た4.2～14.9%．細川ら[9]が東支那海で得た2.0～18.9%などと大差がないように思われる．すでにのべたように，調査水域の中央から浚渫航路入口にいたる一帯（St. 3, 4, 8）では値が小さく，福山入江口（St. 1）と芦田川の河水の影響を受けると考えられるSt. 6, 7では値が大きい．季節変化は明瞭でない.

有機炭素量： 5回の調査で得た測定値は4.3～18.2mg/gで（Table 6），細川ら[9]による東支那海の値1～12mg/g, 加藤[13]による北海道北西海域の値17.4～20.0mg/gなどの範囲にわたっている.

灼熱減量と同様に，水域中央から浚渫航路入口にかけて値が小さく（St. 3, 4, 8），福山入江口（St. 1）と芦田川口に近いSt. 6, 7では値が大きく，季節変化は明瞭でない.

灼熱減量との間に次の直線関係が認められる（Text-fig. 13A）.

$$y = 0.71x - 0.53$$

ただし y：灼熱減量（%），x：有機炭素量（mg/g）.

なお，海底土の風乾試料には海水の塩分が含まれ，その中の塩素が酸化剤を消費するため，有機炭素量の分析値は真の値よりやや大きくなるのが普通である．上にのべた測定値はいずれも，この塩素による誤差を補正してない．調査Vの試料についてこの誤差を測定したところ，有機炭素量として0.8～2.2mg/gであった.

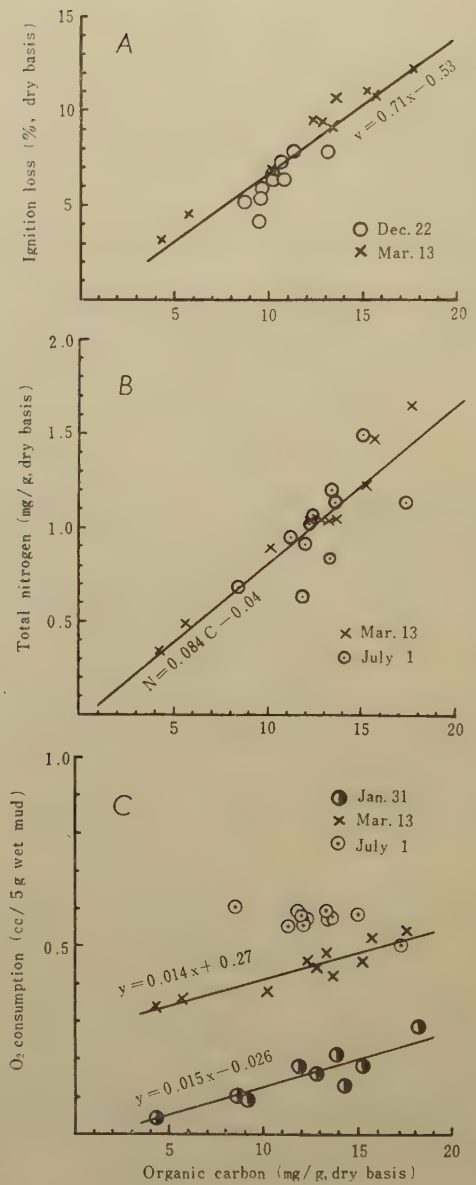
窒素量： 調査は2回行ない，測定値は0.34～1.64mg/gで，細川ら[9]による東支那海の値0.16～1.86mg/gとほぼ似た範囲にあり，加藤[13]による北海道北西海域の値0.6～8.0mg/gより小さい.

調査水域内での分布は灼熱減量・有機炭素量とほぼ同様で，浚渫航路入口付近のSt. 3, 4

Table 6. Results of measurements on the bottom mud.

| Measurement | Date of sampling | Station 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P.V. (cm) | [I*] Oct. 3, 1958 | 4 (3.0) | 34 (4.0) | 7 (4.0) | <33 (4.0) | 34 (3.0) | 49 (5.0) | 47 (6.0) | 45 (…) | 48 (5.0) | 40 (3.5) | … |
| [Sounding (m)] | [II] Nov. 7, 1958 | 45 (5.0) | 27 (4.0) | 27.5 (4.5) | 35 (…) | 33.5 (3.0) | 45 (4.5) | 52 (6.0) | 45 (6.0) | 40 (4.5) | …(4.0) | … |
| | [III] Dec. 22, 1958 | 25 (3.0) | 39 (…) | 33 (5.0) | 34 (4.1) | 33 (4.0) | 49 (5.0) | 48 (5.0) | 51 (6.0) | 51 (5.0) | 48 (5.0) | … |
| | [IV] Jan. 31, 1959 | 35 (3.0) | 33 (2.0) | 15 (2.0) | 30 (2.5) | 34 (2.0) | 52 (4.0) | 53 (4.5) | 37 (4.5) | 50 (4.0) | 63 (3.5) | … |
| | [V] Mar. 13, 1959 | 60 (5.0) | 35 (4.0) | 15 (3.5) | 25 (3.5) | 33 (2.5) | 48 (5.0) | 55 (6.0) | 50 (6.0) | 50 (5.0) | 48 (4.5) | … |
| | [VI] July 1, 1959 | 33 (0.8) | 35 (2.0) | 36 (2.5) | 36 (2.5) | 34 (1.5) | 58 (3.5) | 59 (5.0) | 60 (4.5) | 47 (4.0) | 50 (3.5) | 64 (6.0) |
| Ignition loss | [I] Oct. 3, 1958 | 7.3 | 7.4 | 3.8 | 5.6 | … | … | 9.0 | 6.8 | 8.8 | 6.8 | … |
| (%, dry basis) | [II] Nov. 7, 1958 | 11.3 | 8.4 | 5.6 | 6.3 | 6.9 | 9.7 | 8.3 | 6.7 | 7.4 | 7.4 | … |
| | [III] Dec. 22, 1958 | 5.9 | 6.3 | 5.1 | 5.4 | 6.6 | 7.8 | 7.9 | 4.1 | 6.3 | 7.3 | … |
| | [IV] Jan. 31, 1959 | 12.1 | 7.8 | 3.4 | 6.0 | 9.8 | 10.9 | 10.4 | 6.2 | 9.7 | 8.8 | … |
| | [V] Mar. 13, 1959 | 12.1 | 9.0 | 3.2 | 4.5 | 10.6 | 11.0 | 10.6 | 6.7 | 9.4 | 9.5 | … |
| | [VI] July 1, 1959 | 11.8 | 10.1 | 6.3 | 7.1 | 12.8 | 13.9 | 12.2 | 10.3 | 11.0 | 10.8 | 15.8 |
| Organic carbon** | [II] Nov. 7, 1958 | 15.7 | 11.1 | 9.1 | 9.6 | 10.3 | 15.8 | 13.3 | 12.2 | 11.9 | 11.8 | … |
| (%, dry basis) | [III] Dec. 22, 1958 | 9.6 | 10.8 | 8.7 | 9.5 | 10.2 | 13.1 | 11.3 | 9.5 | 10.1 | 10.6 | … |
| | [IV] Jan. 31, 1959 | 18.2 | 11.9 | 4.3 | 8.6 | 13.9 | 15.3 | 14.3 | 9.2 | 12.8 | 11.8 | … |
| | [V] Mar. 13, 1959 | 17.6 | 13.3 | 4.3 | 5.7 | 15.7 | 15.2 | 13.6 | 10.2 | 12.8 | 12.3 | … |
| | [VI] July 1, 1959 | 13.3 | 11.3 | 8.5 | 11.8 | 15.0 | 13.4 | 13.6 | 12.0 | 12.3 | 12.1 | 17.3 |
| Total nitrogen | [V] Mar. 13, 1959 | 1.64 | 1.03 | 0.34 | 0.48 | 1.46 | 1.21 | 1.04 | 0.89 | 1.04 | 1.03 | … |
| (%, dry basis) | [VI] July 1, 1959 | 0.83 | 0.94 | 0.67 | 0.63 | 1.48 | 1.19 | 1.13 | 0.91 | 1.06 | 1.01 | 1.13 |
| C/N ratio** | [V] Mar. 13, 1959 | 10.7 | 12.9 | 12.6 | 11.9 | 10.8 | 12.6 | 13.1 | 11.5 | 12.3 | 11.9 | … |
| | [VI] July 1, 1959 | 16.0 | 12.0 | 12.7 | 18.7 | 10.1 | 11.3 | 12.0 | 13.2 | 11.6 | 12.0 | 15.3 |
| $O_2$ Consumption | [I] Oct. 3, 1958 | 0.69 | 0.84 | 0.67 | 0.71 | 0.76 | 0.68 | 0.70 | 0.67 | 0.57 | 0.56 | … |
| (cc/5g wet mud) | [II] Nov. 7, 1958 | 0.53 | 0.55 | 0.55 | 0.53 | 0.53 | 0.54 | 0.35 | 0.55 | 0.26 | 0.32 | … |
| | [III] Dec. 22, 1958 | 0.67 | 0.49 | 0.20 | 0.59 | 0.88 | 0.52 | 0.47 | 0.49 | 0.83 | 0.59 | … |
| | [IV] Jan. 31, 1959 | 0.28 | 0.18 | 0.04 | 0.10 | 0.21 | 0.18 | 0.13 | 0.09 | 0.16 | … | … |
| | [V] Mar. 13, 1959 | 0.54 | 0.48 | 0.34 | 0.36 | 0.52 | 0.46 | 0.42 | 0.38 | 0.44 | 0.46 | … |
| | [VI] July 1, 1959 | 0.59 | 0.55 | 0.60 | 0.59 | 0.58 | 0.57 | 0.57 | 0.58 | 0.57 | 0.55 | 0.50 |

* Serial number of the survey. ** Chlorine error not corrected. … Not measured.




Text-fig. 13. Correlation of ignition loss, total nitrogen content and oxygen consumption to organic carbon content in bottom mud.

では値が小さく，福山入江口 (St. 1)，釜屋入江前 (St. 5)，芦田川口に近い St. 6 などでは値が大きい．

有機炭素量との間に次の直線関係が認められる (Text-fig. 13B)．

$$N = 0.084C - 0.04$$

ただし　N：窒素量(mg/g)，C：有機炭素量 (mg/g)．

炭素率 (C/N)：　炭素率 (carbon-nitrogen ratio) は窒素に対する炭素の割合で，陸上土

壌においては，有機物の分解段階・分解速度などの指標として用いられ，その値は10前後の場合が多く，これは有機物の分解が進んだ安定した状態を示す値とされている[18]．一方海底土においても 8～12，平均10の場合が多いといわれている[31]．

2回の調査における測定値は 10.1～18.7 で，異常に高い値（調査 VI の St. A, 1, 4）を除けば，10.1～13.2 の範囲で平均 12.0 である．また調査 V における測定値の平均は 12.0 であるが，塩素誤差を補正すると 10.4 となる．

上記のことから，福山港水域の底質中の有機物は一般によく酸化分解されていると考えられる．しかし，St. 5 および調査 V の St. 1 のように炭素率が小さく有機炭素量・窒素量の大きい，有機物の酸化分解が充分でないと考えられる地点もある．なお，調査 VI において St. A, 1, 4 の値が異常に高かったが，その原因は明らかでない．

底質の酸素消費量：　底質の酸化・還元状態や細菌・酵素などの活性の目安として測定したが，測定方法が適切でなかったために充分な結果は得られなかった．

6回の調査で得た値は 0.04～0.88cc（湿泥5g当り）で範囲が広く，調査のたびに値がかなり変動したが，これは実験温度のちがいによると考えられる．

調査 IV, V における値はかなりよく似ており，その分布は灼熱減量・有機炭素量の分布とほぼ同じ傾向を示す．すなわち，浚渫航路入口および水域中央付近（St. 3, 4, 8）では値が小さく，福山入江口（St. 1），釜屋入江前（St. 5）および St. 2 では値が大きい．

この2回の調査においては，酸素消費量と有機炭素量との間に直線関係が認められた (Text-fig. 13C)．すなわち

$$\text{調査 IV（1月）；}\quad y=0.015x-0.026$$
$$\text{調査 V（3月）；}\quad y=0.014x+0.27$$

ただし　y：酸素消費量（cc/湿泥5g），x：有機炭素量（mg/g）

この $y=ax+b$ という直線関係において，a は 0.014～0.015 でほぼ一定しているが，b は温度の函数と考えられる．調査 I～III, VI において各測点の値が接近したのは，実験温度が高く b の値が大きかったことによると推定されるが，なお検討が必要である．

水質・底質間の関連：　毎回の調査において，福山入江口の St. 1，釜屋入江前の St. 5 などでは灼熱減量・有機炭素量・窒素量・酸素消費量が大きく，これらの測点の底質が有機物に富んでいることが明らかである．これらの測点は，農地排水，都市・工業廃水の影響が強く，水の動きが比較的ゆるやかな所にあたっており，有機質の沈積が多くなっているものと考えられる．

一方，St. 3, 4, 8 などでは有機質含量の指標となる諸量の値が小さいが，これらの地点では水の動きが速く有機質の沈積がさまたげられているものと考えられる．

## (V)　論　　議

### 1.　福山港水域の基本的特徴

前章に詳しくのべたように，福山港水域の海況は区域・季節・潮時により異なり，底質も測点により差がある．しかし，これらの変異ないし多様性の根底に，次のような本海域の基本的特徴を認めることができよう．

① 内湾性の強い水域である．

② 水深が浅く泥底である．

③ 潮差は大きいが潮流は弱い.

④ 陸水の流入量が多くない.

⑤ 汚染度の高い福山入江の水が流入する.

これらの特徴には相互にある程度の因果関係が認められるが，以下それぞれの特徴について考察してみたい.

先ず本水域が内湾性が強いという点は，今回の調査結果において，(a) 水温は年変化の位相が気温のそれと一致し，年較差が大きい，(b) 透明度が小さい，(c) 塩素量が 17‰ 前後である，といった諸点に現われているが，プランクトン[20,21]・底棲生物群集[14,15]の研究においてもすでに指摘されており，後述するようにこの水域の漁獲物組成にも反映されている.

本水域が強い内湾性を示すのは，備後灘自身が内湾的傾向が強いうえに，本水域における局地的条件のためにこの傾向がさらに強められていると考えるが妥当であろう.

備後灘が内湾的性格を示すのは，外洋（太平洋）との間に豊後水道—伊予灘—安芸灘—燧灘（ひうち）が介在するために*，この一連の水域はこの順序に水深[17]（原著 p. 9）・塩分[17]（原著 p. 121–129, 141–147）・透明度[22]が減少し，水温の年較差[17]が増大する．すなわち，備後灘は豊後水道を湾口とする長い入江の奥にあたるわけで，瀬戸内海のうちで最も内湾的傾向の強い水域のひとつとされている[17]．浮遊性毛顎類の組成も豊後水道から備後灘へと次第に内湾的傾向が強まるという[21].

このように備後灘が内湾的傾向が強いうえに，今回の調査水域は陸に近接し水深が浅く，泥底で潮差が大きいため，水温・塩分は気温・陸水の影響を受けやすく，泥粒子の懸濁により透明度が低下し，内湾的傾向が一層強くなっていると考えられる.

本水域が水深が浅く泥底であることは，上記のようにその海況に著しい影響を与えているが，この地形的特徴は芦田川の沖積作用に負う所が多い．すなわち，この付近の海岸がもと沈降海岸として形成された当時は，本土の海岸線（従って芦田川の河口）は現在より数 km 東北方にあり，箕島，福山入江口北岸の丘，皿山などはいずれもその前面に小島として存在した．その後，芦田川の沖積作用によりその付近の水深は次第に浅くなり，特に江戸時代以降には干拓工事が繰返されて海岸線は前進をつづけ，ついに往時の小島を連結する現在の護岸が築造されるに至った.

近年は福山港水域をも埋め立てる計画が立案されており，これに関連して行なわれたボーリング調査[3,8]によれば，海底面から約 10m 下方までは粒子組成がほぼ一様な貝殻まじりのシルト層（粒径 2～20μ の沈泥が 75～90% を占める）で，これは芦田川の沖積作用に海蝕作用が加わって形成されたものとされている．底質が泥質であるのは，その上方の水が多年にわたり懸濁性土粒子に富み，かつ水流が停滞気味であったことを示すと考えられるが，他方，本調査海域のように水深が浅い場合には，いったん海底に沈積した土粒子が風浪などによって再び水中に懸濁し，透明度を減少させ，水域の内湾性を強めるものと考えられる.

海底泥がその上方の海水にいかなる影響を与えているかは興味ある問題であるが，今回の調査ではこの点を充分明かにすることはできなかった．大家[29]は 4～10月の暖季に St. A および10付近で海底面の上方 2cm の水温・塩素量・溶存酸素量を測定し，これを海底上 50cm

* 外洋と備後灘との連絡路としては，この他に，紀伊水道—大阪湾—播磨灘—備讃瀬戸—備後灘があるが，播磨灘がすでに内湾性が強いうえに，備讃瀬戸が浅く狭いから，この経路による外洋水の影響はわずかであろうと考えられる.

または1mの層の値と比較し，潮流の弱い測点では盛夏（7,8月）に海底面上2cm層の酸素量が顕著に少いことを見出し，海底面直上の水層の懸濁質を測定すればさらに興味ある結果が得られるであろうとのべている．

福山港水域の懸濁粒子に関する詳しい調査はまだないが，古川ら[5]（原著 p. 41–44, 136–137）が笠岡湾東部（神島西端と笠岡港を結ぶ線上）で調査した結果によれば，海水1L中に粒径0.4～100$\mu$の粒子が2.0～6.1mg懸濁し，その灰分は69～94%で，海田湾（猿猴川尻），広島湾（太田川尻）にくらべて懸濁物量はやや多く灰分の値は著しく高いという．従って，福山港水域においても，懸濁粒子の量が相当多く，しかも土粒子がその主な部分をしめているのではないかと考えられる．

次に潮差が大きく潮流が比較的弱いという特徴について考えてみよう．すでにのべたように，潮の干満にともなう海況の変化を調査することは，今回の調査の主目的のひとつであった．

平均潮における満・干潮時（潮位はTable 3よりそれぞれ3.17m, 0.99m）の本水域（St. 6～10の線以西）の水量を海図[10]から計算すると，それぞれ$22.7\times10^6m^3$，$11.4\times10^6m^3$となり，満潮時の水量の50%が下げ潮時に水域外へ流れ去る計算となる．大潮，小潮には，この割合はそれぞれ63%，33%となり（潮位はTable 3の大潮差，小潮差の値を用いて計算），本水域では潮汐にともなう水の出入率が非常に大きい．

今回の調査で，本水域では盛夏にも鉛直成層があまり発達せず，また低塩分水の分布が通常陸水の流入口付近に局限されることが確かめられたが，これらは，上記のように水の出入率が高く，したがって擾乱・混合が盛んなことに起因すると考えられる．また，本水域が内湾的傾向が強いにもかかわらず底層水の溶存酸素量があまり低下しないのも，同じ理由によるものと思われる．

水の出入率が高いにもかかわらず潮流が比較的弱いのは，本水域の形態が間口広く奥行小さく海底が平坦であるという特徴を有する上に，その位置が笠岡湾に出入する潮流の主軸からはずれているためである．笠岡湾の潮流は，上げ潮時には主として湾口（箕島－神島間）の東半分をしめる深所を北上し，湾内で次第に右に折れて笠岡の方向にむかい，下げ潮時には湾内各部からほぼ湾口中央部に収れんするような方向に流れるとされており[20]（原著 Fig 40–1～6），このため福山港水域では上げ潮・下げ潮時とも潮流はゆるやかである．

従って，福山港水域では，潮の干満にともなう水の出入は著しいが塩分の高い湾外水が直接流入する機会は比較的少なく，かえって芦田川河口の低塩分水の影響を多く受け，その結果内湾的傾向が強くなるものと考えられる．村上[20]はすでにこの点を指摘し，笠岡湾を東・中・西部に3分した場合，西部（福山港水域）が最も内湾性が強い――塩分が低く透明度が小さく水色が悪く水温の年較差が大きい――としている（原著 p. 41–42）．また，われわれが行なった4回の定期観測において，塩分・透明度の最も高い水が常に沖側中央部のSt. B, 8, 9などに見られたことは上記の潮流のpatternからよく説明される．

次に，陸水の流入量が比較的少ないという特徴は，大きな河川が調査水域内へ放流していないために生じたものであるが，さらに根本的には，この地方の陸水量が少ないこととも関係がある．この特徴は，調査結果の面には，塩素量が比較的高くその年較差が小さく，低塩分水の分布とその底質への影響が陸水流入口付近に局限される，という現象となって現われた．

このように，本水域へ直接流入する陸水の影響は流入口付近に局限されているが，一方，水域外に流入する芦田川の河水の影響が，箕島を迂回して本水域の南半に及んでいる．すなわち，定期観測において箕島東岸（St. 6）に低塩分水が見出され，大雨の後には測点K7に陸水の影響が著しく，また St. 6, 7 の底質には陸水の影響が濃厚である．

最後に，福山港水域の海況に顕著な影響を与えている福山入江の水についてのべる．福山入江および入江口周辺海域の水質・底質の汚染度については，1951, 1953年に新田ら[25]（原著 p. 87-88）が調査し，さらに1954年に北森ら[16]が底棲生物群集を中心に詳しく調査した．これらの調査結果の要点を摘記すれば，

(a) 汚染源は干潮時に入江奥に廃棄される都市・工場廃水である．このため入江奥は最も汚染度が高く，底質は周年黒色で硫化水素臭を帯びる．

(b) 入江の下層を占める海水は潮汐によってある程度更新される．このため底質の汚染度は入江の奥から入江口へ向って軽減する．

(c) 廃水は入江の上層に分布し，干潮時に入江口から海へ流出するが，その影響が顕著に認められる水域は入江口から 500～1,500m の範囲である．

(d) 終戦（1943年）前後には工場廃水の流入がなく，入江口・新涯付近の漁獲量は年37～40トンに達したが，工場の操業再開とともに減少し1953～54年には年15トン程度となった．この場合コノシロ・スズキ・ボラなどの“浮き魚”の漁獲が著しく減少し，ウナギ・アナゴ・エビなど底棲種の漁獲量はあまり減少しなかった．

なお，新田らは上記の調査データを用いて，入江内での浄化速度と酸素のたるみ曲線を計算し[26]（原著 p. 39～43），また入江口周辺の海中における廃水の分散状態[26]（原著 p. 30）および廃水の影響範囲[27]（原著 p. 130, 135）を論じた．

その後，1958年9月の調査*では，入江の水質は1953年よりかなり改善されており，入江奥の C.O.D. は $\frac{1}{10}$ 以下に減少していたという。

一方，藤山[1,2]，片山ら[12]は福山入江口のノリ養殖場に発生するアサクサノリの癌腫病について報告し，調査を行なった1952～57年には毎年規則正しく発生し，病徴の顕著な漁場は入江口から 500～700mの範囲であったとして（Text-fig. 14），本病を誘発する発癌物質は入江奥に廃棄される都市・工場廃水中に含まれているものと推定した．

## 2. 生物群集および漁業

福山港水域の生物群集や漁業は，当然，上に考察した5つの特徴を有するこの水域の海況・底質によって性格ずけられていると考えられる．しかしわれわれはこの点に関してまだ充分な資料を集積していないので，ここでは既刊の業績・資料を列挙し多少の考察を加えるにとどめる．

先ずプランクトンについては，村上が笠岡湾の浮遊性珪藻群集の年変化を概説し[20]，また笠岡湾および近海の浮遊性毛顎類の生態を詳しく報告している[21]．弓場[35]は定期観測IIにさいし各測点の1m層のミクロプランクトンを調査し**，全般的に珪藻が主体を占めるが，福山入江・釜屋入江付近の低塩分域には *Tintinnopsis kofoidi*[6] が，沖側の高塩分域には *Ceratium tripos* が分布し，中間的な塩分を示す水域には両者が混在することを見出し，本水域内の水系区分にはミクロプランクトンが指標として役立つことを示唆した．

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* 新田忠雄・他：福山港附近の水質について．（内水研利用部資料，未発表）．

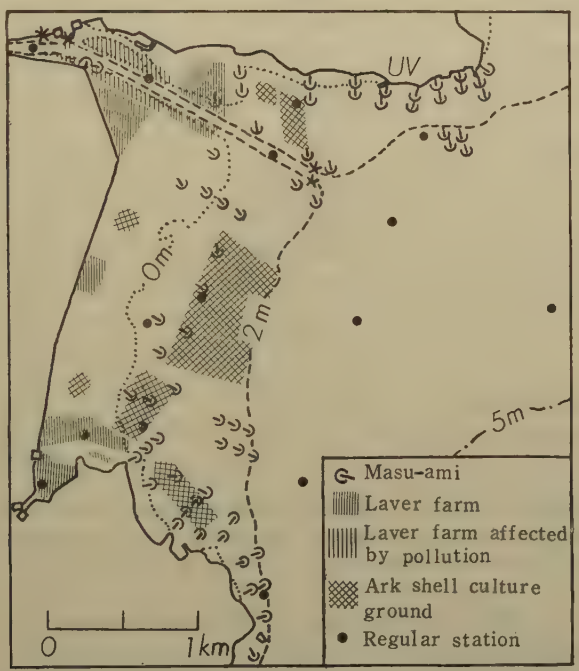
** 採水・沈でん法による．

底棲群集動物については，1943年秋に川口・白井[14]が，翌年春に川口[15]がそれぞれ笠岡湾全域の41測点で調査し，二枚貝を主体とする強内湾性の群集が見られることを報告している．

なおここでアマモ *Zostera marina* のことにふれておきたい．1953年および1955年に本水域の水質・底質などを調査した新田[26]（原著 p. 26–32）および大家[29]によれば，当時は2m等深線以浅の測点ではほぼ例外なくアマモの群落が見られたようである．しかし，われわれが1958～60年に今回の海況調査を行なった時には，アマモは釜屋入江前付近にわずかに見られただけで，採水・採泥にさいしても生葉・枯葉がロープや測器にからまったことはなく，漂流中の葉を認めることもまれであった．桝網業者によれば，1961年にはアマモが網に流入することはほとんどなかったという．以上の情報はいずれも数量的な厳密性を欠くが，本水域のアマモが1950年代後半の数年間に消滅に近いまでに減少したのではないかと考えられる．

次に本水域で行われている漁業にふれておく．おもな漁業は，桝網漁業，ノリ養殖，モガイ養殖であり，このほかマハゼ・イイダコなどの遊漁が多少行なわれ，岸ぞいの干潟で天然産のアサリ，餌虫が採捕される．

桝網は陸側から沖へ向って張り出した垣網（長さ40m内外）とその沖端にある6角形の身網（間口 25m，奥行 13m）からなる小型定置網で，海中に立てた竹の支柱の列にとりつける．主として0,2m等深線間に設置され，潮流にしたがって移動する魚類・エビ・カニ・イ




Text-fig. 14. Location of pound nets ("masu-ami"), laver farms and ark shell culture grounds in Fukuyama Herbor in October, 1961.
UV……Faculty campus
0m, 2m, 5m……Depth below the lowest low water level.

カ類が漁獲される．盛漁期の4～10月には福山港水域内で約70統が操業する（Text-fig. 14）．Table 7 に示した調査水域内の漁獲物は，養殖によるものおよびアサリを除けば，ほとんどすべて桝網によるもので，内湾性の強い魚種組成ということができよう．

Table 7. Landing from Fukuyama Harbor in 1959*

| Scientific or English name | Japanese name | Landing in metric tons** | Type of fishery*** |
|---|---|---|---|
| **Marine animals, total** | **Dobutsu gokei** | **956.5** | |
| **Fishes** | **Gyorui** | **53.6** | |
| *Mugil cephalus* | Bora | 8.0 | M |
| *Lateolabrax japonicus* | Suzuki | 7.1 | M |
| *Sparus swinhonis* | Kurodai | 6.8 | M |
| *Clupanodon punctatus* | Konoshiro | 4.8 | M |
| Flounders | Karei rui | 1.5 | M |
| *Harengula zunasi* | Sappa | 1.3 | M |
| Lizard fish | Eso | 1.2 | M |
| Puffers | Fugu rui | 1.2 | M |
| *Nibea argentata* | Ishimochi | 1.1 | M |
| Other fishes | | 20.6 | M |
| **Molluscs** | **Kairui ika tako** | **889.8** | |
| *Venerupis semidecussata* | Asari | 390.4 | O |
| *Anadara subcrenata* | Mogai | 333.2 | C |
| *Meretrix meretrix lusoria* | Hamaguri | 12.7 | O |
| Other shellfishes | Kai rui | 141.1**** | O |
| Octopus | Tako | 8.7 | M |
| Cuttlefishes and squids | Ika | 3.7 | M |
| **Crustaceans** | **Ebi kani** | **13.1** | |
| *Penaeus japonicus* | Kuruma-ebi | 2.2 | M |
| Other shrimps | Ebi rui | 6.5 | M |
| Crabs | Kani | 4.4 | M |
| **Seaweeds, total** | **Kaiso rui** | **1.81♯** | |
| *Porphyra tenera* | Kuro-nori | 1.07♯ | C |
| *Enteromorpha* and *Monostroma* | Ao-nori | 0.08♯ | C |
| *Porphyra* mixed with *Enteromorpha* or *Monostroma* | Maze-nori | 0.66♯ | C |

* Total landing by the Fukuyama, Hikino and Otsuno Fishing Cooperatives, whose fishing and culture grounds approximately coincide the area enclosed with thick broken line in Text-fig. 1. (Source : Hiroshima Statistical Survey Office, Ministry of Agriculture and Forestry[25]).

** Gross weight unless otherwise stated.

*** Type of fishery : C - culture, M-"masu-ami" or pound net, O - others.

**** Includes 3 tons of sea-cucumber and sea-urchin.

♯ Dry weight. Calculated with the conversion factor of 1000 sheets of dried laver＝2.3kg.

ノリ養殖場は陸水の流入口付近に，モガイの養殖場はその沖側に設けられ（Text-fig. 14），その産額は Table 7 に示した通りである．新涯沖の2m等深線付近の広いモガイ養殖場は採

苗に適している[7].

### 3. 観測方法について

最後に，今後今回のような目的をもって調査を行なう場合を仮定して，観測方法の問題にふれておきたい．今回実施した4回の定期観測の主な目的は，面積の比較的限られた沿岸水域の海況が潮汐にともなってどのように変化するかを明らかにすることであった．この目的に対しては，(a) 測点間隔をさらにちぢめ対象水域外にも測点をおくことが望ましく，(b) そのためには測器の改善，艇速の増加などにより測点当りの観測所要時間を短縮する必要があろう．また (c) 主要測点に連続的自記測器を定置し，(d) 水系の判別を明確にするためにプランクトン相，濁度の観測を追加することが望ましい．

また水域内の各部分の海況の特性を知るためには，底質の理化学的調査のほかに底棲生物群集を調査すれば効果が大きいと考えられる．

## (VI) 要　　約

福山港水域の海況を明かにする目的で，1958年9月から1959年7月にかけて定期海況観測を4回，底質調査を6回行ない，さらにこれらを補足するため，1960年7月に12時間連続観測および流入陸水調査を行ない，この水域の海況および底質について次の諸点を明かにした．

海況については

(1) 福山港水域は，内湾である笠岡湾の一部で面積約6.1 km$^2$，水深は浅く干潟をもち，平均潮における満潮時の水量は $23\times10^6$ ton である．水域の北西および南西部には都市・工業廃水，農地排水の流入がみられる．潮差が比較的大きく，満潮時の水量の33～63%が下げ潮時に水域外へ流出する．

(2) このように水の出入率は高いが，潮流は比較的ゆるやかである．これは，調査水域の地形が開口が広く奥行が浅く，かつ海底が平坦なことによると考えられる．水の動きとしては，上げ潮時に沖合水はこの水域の東側中央にあたる St. 8 付近から浚渫航路に向って差し込む傾向を示し，下げ潮時には北西の福山入江口，南西の釜屋入江口を中心に陸水の影響の強い水が表層（1m以浅）にひろがる．このほか，芦田川口起源と考えられる低塩分水が南西部（St. 6 付近）の表層に出現する場合が多い．

(3) 水温は，周年変化が大きく，月平均水温の最高は28°C．最低は約7°Cである．気温との差は小さく，従って気温の影響を強く受けていると考えられる．

(4) 塩素量は，陸水流入口付近を除けば，周年ほぼ16～18‰の範囲である．その空間的分布は沿岸・表層で低く，沖合・底層で高い傾向を示すが，陸水の影響を強く受ける入江口付近（St. A, 1,5 など）を除けば，比較的均一である．また入江口付近でも満潮時には流入する沖合水の影響が卓越する．

(5) 溶存酸素量は，周年比較的多く，飽和に近いか過飽和の場合が多い．分布は，沿岸・底層に少なく沖合・表層に多い傾向を示し，陸水ことに福山入江の水が混入した場合は酸素量が少ない．また局所的に酸素量が多い場合が見られ，これは植物プランクトンの濃群によると思われる．

(6) C.O.D. は，大部分の地点で0.6～1.9 ppm 位である．陸水の影響の強い水ではこれより大きく，最大6 ppm を観測した．

(7) 酸可溶性全鉄量は一般に 0.05 ppm 以下であるが，底層水ではしばしばこれより大きく，陸水の影響の強い水ではさらに大きく最大 0.6 ppm を観測した．

(8) pH は，一般に 8.2 であるが陸水の影響の強い水ではこれより小さく，最低 7.2 を観測した．

(9) 水色は 4～9 で，沿岸では悪く，沖合では比較的よく 4～6 である．

(10) 透明度は 1～4m で，沿岸で小さく沖合で大きい．

(11) 以上のことから，福山港水域の水は 3 系統に大別できると考えられる．すなわち

① 陸水系の水は，塩素量・溶存酸素量が小さく，C. O. D. ・鉄量が大きく，pH は低く，水色悪く透明度が小さい．

② 沖合系の水は，塩素量・溶存酸素素量が大きく，C. O. D. ・鉄量は小さく，pH は高く (8.2～8.3)，水色は 5，透明度が大きい．

③ 底層系の水は，塩素量は大きく 17‰ 以上，溶存酸素量は表層よりは小さく，鉄量は表層より多い．

これら 3 系統の水が潮汐による水の流動によって混合・交代し，これに大気・日射の影響，生物・懸濁質の生化学的作用などが加わって複雑な様相を呈すると考えられる．

底質については

(12) 底質は軟泥質で粒径 2～20$\mu$ の沈泥が 75～90% を占める貝殻まじりのシルトからなり，灼熱減量 3.2～13.9%，有機炭素量 4.3～18.2mg/g，窒素量 0.34～1.64mg/g であった．灼熱減量・窒素量と有機炭素量の間には直線関係が認められる．塩素の補正を行った炭素率 (C/N) は 10 前後の場合が多いから，底質中の有機物の酸化分解はかなり進んでいると考えられる．

(13) 酸素消費量は，測定方法に欠陥があったが，0.04～0.88cc (湿泥5g当り) の範囲で，比較的低温で測定した場合 (底質調査 IV, V) には有機炭素量との間に直線関係が見られた．

(14) 貫入深度はほぼ 2m 等深線を境にしてかなり差がある．すなわち，2m 以浅の海域では 30cm 前後，以深の海域で 50cm 前後である．

(15) 全般的な傾向としては，陸水の影響を強く受ける測点 (St. 1, 5, 6 など) の底質は灼熱減量・有機炭素量・窒素量・酸素消費量が大きく炭素率は小さい．すなわち，有機質に富み，その分解が充分に進んでいない．一方，水の動きが比較的大きい測点 (St. 3, 4, 8 など) では有機質に乏しく貫入深度が小さい．

以上の知見を既往の海況・底質・生物群集・漁業に関する資料と比較して論議した．

## SUMMARY

A series of hydrographic observations were carried out from September, 1958 through July, 1960 in order to clarify the physical and chemical properties of sea water and bottom muds of Fukuyama Harbor, Hiroshima Pref. (Text-figs. 1,2, Table 4). The results are presented in Tables 6, A-F and Text-figs. 4–12 and discussed in comparison with previously published data on the hydrography, ecology and fishery of this water area.

Fukuyama Harbor* is the former estuary of Ashida River. It occupies the western part of Kasaoka Bay and measures 6.1km$^2$ in area. Being trapezoidal in shape, it is bordered by a reclaimed land in the west, by hilly peninsula in the south and north, and opens wide in the east. The flat muddy bottom slopes down gently toward the east. Water depth does not exceed 7m below the mean sea level at any part of the harbor. Tidal flats are exposed along the shore at low waters.

Water temperature differs very little from air temperature all the year, the monthly mean varying between 7° (January and February) and 28°C (August) at the surface (Text-fig. 3). Water is turbid with the Secchi disc depth seldom exceeding 4 m. These two features can be ascribed partly to such local conditions as the small water depth and the muddy bottom, but are principally due to the fact that the water can not freely pass from the high sea (i. e., the Pacific Ocean off Bungo Strait) to this locality owing to the channels and shallow seas lying on the course.

Monthly mean of chlorinity varies within the range of 16.6–17.9‰ at the surface (Text-fig. 3). Seasonal variation of chlorinity is not very great and reaches minima in summer when local precipitation and the drainage of Ashida River reach maxima (Tables 1, 2).

Tidal range is comparatively great owing to the interference of the two tidal waves traversing the Seto Inland Sea in opposite directions, one wave from Bungo strait toward the east and the other from Kii Strait toward the west. In an average tide, tidal range measures 2.2m (Table 3) and 50% of the water that is present in the harbor at the high water is drained off during the ebb. Since water is mixed and replaced by the tide, vertical stratification seldom develops and the water is rich in dissolved oxygen from surface to bottom throughout the seasons. Tidal current, however, is not very fast.

At low tides C.O.D. increases and dissolved oxygen decreases in the water in the northwestern part of the harbor, where polluted water is discharged from Fukuyama Inlet. In rainy months fresh water is discharged from flood-gates at low tides, temporarily lowering the chlorinity of the surface layer nearby.

In the area where the effect of the polluted water or fresh water is not appreciable, various measurements on water are generally within the following range: water color in Forel's scale, 5 or more; pH, 8.2–8.3; C.O.D. by Saeki's alkaline permanganate method[30], 0.9–1.9ppm; acid-soluble total iron by $\alpha\alpha'$-dipyridyl method, less than 0.05ppm. The water near the sea bottom often gives greater values of C.O.D. and acid-soluble total iron than those mentioned above.

The bottom mud is principally composed of the silt of particle diameters between 2 and 20$\mu$. The bottom is harder on the inshore side of the 2m depth contour than on the offshore side: the penetration value obtained with the Furukawa's penetrometer[4] averages 30 and 50cm respectively. Other measurements on the bottom

* "Fukuyama Harbor" is a provisional name with which the present authors designate the water area under consideration. This area is not used as a refuge by ships because the water is too shallow, although it forms the outer part of Fukuyama Port. The word of 'harbor' is used simply in the sense of 'a sheltered arm of the sea'.

mud are within the following range: ignition loss, 3.2–13.9%; organic carbon by Tiurin's rapid titration method, 4.3–18.2mg/g (chlorine error not corrected); total nitrogen by Kjeldahl method, 0.34–1.64mg/g (Table 6). Carbon-nitrogen ratio (chlorine error corrected) is usually close to 10. Ignition loss, Kjeldahl nitrogen and the oxygen consumption of mud (measured at room temperatures) respectively hold linear relation with organic carbon content (Text-fig. 13).

Bottom mud is rich in organic matter and gives small carbon-nitrogen ratio in the area affected by the drainage from Fukuyama Inlet, flood-gates or Ashida River.

Major fisheries in Fukuyama Harbor are the "masu-ami" fishery and the culture of the ark shell (*Anadara subcrenata*) and the laver (*Porphyra tenera*). The "masu-ami" is a pound net consisting of a pound 25m wide and 13m long and a leader net about 40m long. It is usually set between the 0 and 2m depth contours, being held in place by bamboo poles driven into the bottom (Text-fig. 14). Fishes, crustaceans and cephalopods are trapped in it as they move with the tide. In 1959 about 70 nets were operated in Fukuyama Harbor with the total catch of 79 metric tons. In the same year, 333 tons of ark shell and 1.8 tons of dried laver were produced by culture and 390 tons of littleneck clam (*Venerupis semidecussata*) were harvested from the natural beds on tidal flats (Table 7). All the species represented in the commercial catch are typical inhabitants of such inshore waters where water is relatively turbid and seasonal variation of water temperature is great.

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## Appendix: Data of Hydrographic Observations

Table A. Data of "Regular Observation I".
1. Early ebb (morning) : September 24, 1958.
2. Early flood (afternoon) : September 24, 1958.

Table B. Data of "Regular Observation II".
1. High water (morning) : November 10, 1958.
2. Late ebb (afternoon) : November 10, 1958.

Table C. Data of "Regular Observation III".
1. Flood (morning) : March 13, 1959.
2. Ebb (afternoon) : March 13, 1959.

Table D. Data of "Regular Observation IV".
1. Ebb (morning) : July 1, 1959.
2. Early flood (afternoon) : July 1, 1959.

Table E. Data of "12-Hour Observation" at St. T on July 10, 1960.

Table F. Data of "Land Drainage Survey" on July 11, 1960.

### Abbreviations and Marks Used in Tables A–F.

br……brownish.
$C_F$……Water color in Forel's scale.
Cl……Chlorinity.
COD……Chemical oxygen demand by alkaline $KMnO_4$ method.
d……darkish.
Ex. coef. 0–1m……Extinction coefficient computed from the illuminations immediately (0m) and 1m below sea surface. Illumination was measured with a selenium photocell (maximum sensitivity at 580m$\mu$) equipped with a white diffusing plate.
Fe……Iron.
$O_2$ (cc/L)……Dissolved oxygen in volume as reduced to the stansard state.
$O_2$ (%)……Saturation percentage of dissolved oxygen.
Tr……Transparency measured with the white Secchi disc 30cm in diameter.
$\theta_A$……Air temperature 1.2m above the sea surface.
$\theta_W$……Water temperature.
Z……Observation depth.
… Not measured.
Wind……Direction and the wind force in Beaufort's scale.

Table A–1. Early ebb (morning) : September 24, 1958

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr(m) & $C_F$ | Surface Current (cm/sec) | Weather, $\theta_A$(°C) & Wind |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 25.0 | 16.47 | 19.4 | 8.2 | 3.87 | 76.0 | 0.14 | 1.7 | 1.7 | ESE | Cloudy |
| 09h12m | 1 | 25.6 | 16.82 | 19.7 | 8.2 | 3.94 | 78.5 | 0.07 | 1.2 | 6d | 5 | 22.5 |
| 5.5m | 4 | 25.4 | 17.03 | 20.1 | 8.2 | 3.67 | 73.0 | 0.16 | 1.4 | | | N–1 |
| **2** | 0 | 24.8 | 16.84 | 20.0 | 8.2 | 4.25 | 83.7 | <0.05 | 1.3 | 2.0 | ESE | … |
| 09h56m | 1 | 25.6 | 16.86 | 19.8 | 8.2 | 4.32 | 86.1 | <0.05 | 1.5 | … | 10 | … |
| 4.5m | 3 | 25.6 | 17.14 | 20.2 | 8.2 | 3.86 | 77.2 | 0.06 | 1.8 | | | … |
| **3** | 0 | 25.2 | 16.84 | 19.9 | 8.2 | 4.50 | 89.1 | <0.05 | 1.5 | 1.9 | NW | Cloudy |
| 10h05m | 1 | 25.5 | 16.78 | 19.7 | 8.2 | 4.36 | 86.7 | <0.05 | 1.4 | 4～5d | 10 | 22.5 |
| 7.0m | 5.5 | 25.6 | 17.10 | 20.1 | 8.2 | 3.72 | 74.4 | 0.05 | 1.5 | | | N–1 |
| **4** | 0 | 24.6 | 16.52 | 19.6 | 8.2 | 4.59 | 91.4 | 0.06 | 1.5 | 2.2 | ESE | Cloudy |
| 10h23m | 1 | 25.2 | … | … | 8.2 | 4.50 | … | <0.05 | … | 5d | 19 | … |
| 5.0m | 3.5 | 25.2 | 16.99 | 20.1 | 8.2 | 4.54 | 90.1 | <0.05 | 1.5 | | | NW–1 |
| **5** | 0 | 24.8 | 16.25 | 19.2 | 8.2 | 4.55 | 88.9 | 0.05 | 1.3 | 2.0 | SE | … |
| 10h36m | 1 | 24.9 | 16.21 | 19.1 | 8.2 | 4.37 | 85.7 | 0.05 | 1.6 | 5 | 17 | … |
| 4.0m | 3 | 25.1 | 16.95 | 20.0 | 8.2 | 4.31 | 85.3 | 0.06 | 1.5 | | | NW |
| **6** | 0 | 24.8 | 16.10 | 19.0 | 8.2 | 4.61 | 89.9 | 0.10 | 1.3 | 1.9 | E | … |
| 10h51m | 1 | 25.8 | 17.01 | 19.9 | 8.2 | 4.52 | 90.6 | <0.05 | 1.1 | 5d | 12 | 23.5 |
| 5.0m | 4 | 25.3 | 17.04 | 20.1 | 8.2 | 4.49 | 89.3 | 0.05 | 1.3 | | | … |
| **7** | 0 | 25.0 | 16.76 | 19.8 | 8.2 | 4.70 | 92.7 | <0.05 | 1.5 | 6.0 | N | … |
| 11h05m | 1 | 25.3 | 16.77 | 19.8 | 8.2 | 4.80 | 95.2 | <0.05 | 0.9 | … | 9 | … |
| 6.0m | 5 | 25.3 | 17.03 | 20.1 | 8.2 | 4.43 | 88.1 | 0.05 | 1.4 | | | NE–2 |
| **8** | 0 | 25.0 | 16.97 | 20.1 | 8.2 | 4.76 | 94.1 | <0.05 | 1.3 | 5.5 | NNE | 22.0 |
| 11h16m | 1 | 25.4 | 16.96 | 20.0 | 8.2 | 4.96 | 98.6 | <0.05 | 0.9 | 5d | 16 | Cloudy |
| 5.5m | 4.5 | 25.1 | 17.05 | 20.2 | 8.2 | 4.33 | 85.7 | 0.05 | 1.4 | | | NNE–1~2 |
| **9** | 0 | 24.8 | 16.95 | 20.1 | 8.2 | 4.71 | 92.7 | <0.05 | 1.5 | 6.0 | NE | … |
| 11h27m | 1 | 25.5 | 16.92 | 19.9 | 8.2 | 4.66 | 93.0 | <0.05 | 1.4 | 4～5d | 8 | … |
| 6.0m | 4.5 | 25.4 | 17.10 | 20.2 | 8.2 | 4.16 | 82.9 | 0.20 | 1.5 | | | ENE–2 |
| **10** | 0 | 25.0 | 16.99 | 20.1 | 8.2 | 4.43 | 88.0 | <0.05 | 1.7 | 4.5 | ESE | … |
| 11h42m | 1 | 25.6 | 16.98 | 19.9 | 8.2 | 4.53 | 90.6 | <0.05 | 1.3 | 5d | 7 | … |
| 4.5m | 3 | 25.7 | 17.15 | 20.1 | 8.2 | 3.68 | 74.0 | 0.10 | 1.4 | | | … |

Table A-2. Early flood (afternoon) : September 24, 1958

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr(m) & $C_F$ | Surface Current (cm/sec) | Weather, $\theta_A$(°C) & Wind |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 24.8 | 7.35 | 7.1 | 7.2 | 3.46 | 61.5 | 0.38 | 4.4 | ··· | SSW | Cloudy |
| 16$^h$59$^m$ | 1 | 26.2 | 16.76 | 19.4 | 8.2 | 3.33 | 67.0 | 0.18 | 1.4 | 9 | 4 | 23.1 |
| 1.5m | | | | | | | | | | | | NW/W-1 |
| **2** | 0 | 25.8 | 16.27 | 18.9 | 8.2 | 3.61 | 71.6 | 0.07 | 1.2 | ca.1.5 | NW | ··· |
| 16$^h$44$^m$ | 1 | 26.2 | ··· | ··· | 8.2 | ··· | ··· | ··· | ··· | 7 | 4 | 24.2 |
| 2.5m | 1.5 | 25.6 | 17.07 | 20.1 | 8.2 | 3.43 | 68.6 | 0.05 | 1.3 | | | 0 |
| **3** | 0 | 25.6 | 15.41 | 18.8 | 8.2 | 3.61 | 71.3 | 0.15 | 1.7 | ··· | E | Cloudy |
| 16$^h$34$^m$ | 1 | 26.1 | 17.03 | 19.8 | 8.2 | 3.48 | 70.2 | 0.11 | 1.8 | 6～7d | 4 | 24.4 |
| 2.5m | 1.5 | 25.7 | 17.02 | 20.0 | 8.2 | 3.43 | 68.6 | 0.13 | 1.7 | | | 0 |
| **4** | 0 | 25.2 | 14.63 | 16.9 | 8.2 | 4.04 | 78.0 | 0.12 | 2.1 | 1.7 | W | Cloudy |
| 16$^h$23$^m$ | 1 | 25.8 | 16.73 | 19.5 | 8.2 | 4.57 | 91.4 | <0.05 | 1.6 | ··· | 4 | 25.0 |
| 3.0m | 2 | 25.4 | 16.88 | 19.9 | 8.2 | 4.08 | 81.1 | 0.11 | 1.6 | | | 0 |
| **5** | 0 | 25.2 | 14.56 | 16.8 | 8.2 | 4.80 | 92.7 | 0.12 | 2.1 | ··· | WNW | Cloudy |
| 16$^h$06$^m$ | 1 | 25.6 | 16.80 | 19.7 | 8.2 | 4.07 | 81.1 | 0.23 | 1.2 | 8～9br | 4 | 24.3 |
| 2.0m | | | | | | | | | | | | N/W-1 |
| **6** | 0 | 25.0 | 16.35 | 19.3 | 8.2 | 5.15 | 101.0 | <0.05 | 1.8 | 1.9 | E | ··· |
| 15$^h$50$^m$ | 1 | 25.6 | 16.90 | 19.8 | 8.2 | 4.55 | 90.8 | <0.05 | 0.9 | 7d | 12 | 24.2 |
| 4.5m | 3.5 | 25.5 | 17.03 | 20.0 | 8.2 | 4.16 | 83.0 | 0.18 | 1.5 | | | N/W-1 |
| **7** | 0 | 25.4 | 16.95 | 19.9 | ··· | 5.34 | 106.2 | 0.05 | 1.5 | 2.6 | N | ··· |
| 15$^h$31$^m$ | 1 | 25.5 | 16.93 | 19.9 | ··· | 5.08 | 101.2 | 0.05 | 1.8 | 7d | 8 | 25.8 |
| 5.0m | 3.5 | 25.4 | 16.98 | 20.0 | ··· | 4.83 | 96.0 | 0.05 | 1.3 | | | N/W-1 |
| **8** | 0 | 25.6 | 16.92 | 19.8 | 8.2 | 5.07 | 101.2 | <0.05 | 1.2 | 2.0 | NE/E | ··· |
| 15$^h$17$^m$ | 1 | 25.7 | 16.96 | 19.9 | 8.2 | 4.93 | 98.6 | <0.05 | 1.6 | 7d | 5 | 23.8 |
| 5.0m | 4 | 25.6 | 17.06 | 20.0 | 8.2 | 4.32 | 86.4 | 0.07 | 1.4 | | | N/W-1 |
| **9** | 0 | 25.4 | 16.01 | 18.7 | 8.2 | 4.44 | 87.7 | 0.13 | 1.8 | 1.9 | NE | Fair |
| 15$^h$02$^m$ | 1 | 26.0 | 17.00 | 19.8 | 8.2 | 4.71 | 94.8 | 0.05 | 1.6 | 6～7d | 10 | ··· |
| 4.5m | 3.5 | 25.6 | 17.10 | 20.1 | 8.2 | 3.84 | 76.8 | 0.20 | 2.1 | | | NE-1 |
| **10** | 0 | 25.6 | 17.05 | 20.0 | 8.2 | 4.90 | 98.0 | 0.05 | 1.7 | 1.9 | NEE | ··· |
| 14$^h$45$^m$ | 1 | 25.8 | 16.98 | 19.9 | 8.2 | 4.97 | 99.6 | 0.06 | 2.0 | 5～6d | 4 | 23.2 |
| 4.0m | 2.5 | 26.0 | 17.16 | 20.1 | 8.2 | 4.06 | 81.7 | 0.16 | 1.8 | | | 0 |

Table B-1. High water (morning) : November 10, 1958

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr(m) & $C_F$ | Weather, $\theta_A$ (°C) & Wind |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 17.4 | 16.41 | 21.4 | 8.1 | 4.84 | 83.6 | <0.05 | 1.63 | 2.0 | … |
| 09h50m | 1 | 17.9 | 16.89 | 21.9 | 8.1 | 5.06 | 88.6 | <0.05 | 1.17 | 7 | … |
| 5.1m | 4.5 | 17.6 | 17.09 | 22.2 | 8.1 | 5.16 | 90.1 | <0.05 | 1.36 | | 0 |
| **2** | 0 | 17.6 | 16.95 | 22.1 | 8.1 | 5.13 | 89.4 | <0.05 | 1.34 | 2.5 | Fair |
| 10h04m | 1 | 17.7 | 16.96 | 22.0 | 8.1 | 5.15 | 89.9 | <0.05 | 1.55 | 7 | 18.4 |
| 4.9m | 4.5 | 18.4 | 17.10 | 22.1 | 8.1 | 5.28 | 93.5 | <0.05 | 1.29 | | 0 |
| **3** | 0 | 17.5 | 16.99 | 22.1 | 8.1 | 5.28 | 92.0 | <0.05 | 1.17 | 2.7 | Fair |
| 10h10m | 1 | 17.9 | 17.01 | 22.1 | 8.1 | 5.31 | 93.2 | <0.05 | 1.48 | 7 | … |
| 5.0m | 4.5 | 18.2 | 17.14 | 22.1 | 8.1 | 5.20 | 91.9 | <0.05 | 1.37 | | … |
| **4** | 0 | 17.3 | 16.87 | 22.0 | 8.1 | 5.41 | 93.9 | 0.05 | 1.29 | 2.6 | … |
| 10h23m | 1 | 17.9 | 17.15 | 22.3 | 8.1 | 5.42 | 95.3 | 0.05 | 1.26 | 7 | 17.2 |
| 5.0m | 4.5 | 18.2 | 17.19 | 22.2 | 8.1 | 5.35 | 94.2 | 0.06 | 1.48 | | … |
| **5** | 0 | 17.5 | 16.74 | 21.8 | 8.2 | 5.36 | 92.7 | <0.05 | 1.76 | 2.1 | Fair |
| 10h58m | 1 | 17.8 | 16.95 | 22.0 | 8.2 | 5.43 | 95.1 | <0.05 | 1.31 | 7 | 17.4 |
| 4.7m | 4.0 | 18.2 | 17.04 | 22.2 | 8.2 | 5.36 | 94.5 | <0.05 | 1.45 | | 0 |
| **6** | 0 | 17.5 | 15.65 | 20.5 | 8.2 | 5.61 | 96.2 | <0.05 | 1.48 | 2.1 | Fair |
| 11h12m | 1 | 17.8 | 17.05 | 22.4 | 8.2 | 5.35 | 93.7 | <0.05 | 1.61 | 8 | 20.3 |
| 6.0m | 5.5 | 17.9 | 17.17 | 22.5 | 8.2 | 5.34 | 93.8 | 0.31 | 1.69 | | 0 |
| **8** | 0 | 18.2 | 17.08 | 22.1 | 8.2 | 5.47 | 96.5 | <0.05 | 1.02 | 3.2 | Fair |
| 11h30m | 1 | 18.2 | 17.09 | 22.1 | 8.2 | 5.49 | 96.8 | <0.05 | 1.09 | 6 | 22.0 |
| 6.0m | 5.5 | 18.1 | 17.19 | 22.3 | 8.2 | 5.27 | 92.9 | 0.11 | 1.69 | | 0 |
| **10** | 0 | 18.1 | 17.07 | 22.1 | 8.2 | 5.29 | 93.4 | <0.05 | 1.47 | 3.0 | … |
| 11h58m | 1 | 18.4 | 17.00 | 21.9 | 8.2 | 5.35 | 94.7 | <0.05 | 1.33 | 6 | … |
| 5.0m | 4.5 | 18.1 | 17.18 | 22.2 | 8.2 | 5.22 | 92.1 | 0.09 | 1.39 | | … |
| **A** | 0 | 17.2 | 15.98 | 20.8 | 8.2 | 4.53 | 77.4 | 0.15 | 1.56 | 1.8 | Fair |
| 09h32m | 1 | 17.2 | 16.91 | 22.1 | 8.2 | 4.71 | 81.5 | 0.05 | 1.36 | 7d | 16.9 |
| 4.0m | 3.5 | 17.5 | 17.02 | 22.2 | 8.1 | 4.89 | 84.1 | 0.08 | 1.48 | | 0 |
| **B** | 0 | 18.8 | 17.24 | 22.2 | 8.2 | 5.37 | 95.7 | <0.05 | 1.01 | 3.5 | … |
| 11h44m | 1 | 18.5 | 17.18 | 22.2 | 8.2 | 5.30 | 94.1 | <0.05 | 1.28 | 6 | 20.6 |
| 8.0m | 7.5 | 18.5 | 17.25 | 22.2 | 8.2 | 5.19 | 92.2 | <0.05 | 1.04 | | … |
| **C** | 0 | 17.4 | 16.74 | 21.8 | 8.2 | 5.47 | 94.8 | <0.05 | 1.39 | 2.3 | … |
| 10h32m | 1 | 17.4 | 17.00 | 22.2 | 8.2 | 5.44 | 94.6 | <0.05 | 1.72 | … | … |
| 4.7m | 4.0 | 17.7 | 17.06 | 22.2 | 8.2 | 5.19 | 90.7 | 0.05 | 2.04 | | … |
| **E** | 0 | 17.5 | 14.84 | 19.2 | 8.1 | 4.68 | 79.5 | 0.06 | 1.69 | 2.1 | Fair |
| 10h45m | 1 | 17.7 | 16.53 | 21.5 | 8.1 | 4.86 | 84.2 | <0.05 | 1.64 | 8~9 | … |
| 4.0m | 3.5 | 17.8 | 16.79 | 21.8 | 8.1 | 5.08 | 88.3 | 0.18 | 1.72 | | 0 |

Table B-2. Late ebb (afternoon) : November 10, 1958

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr(m) & $C_F$ | Weather, $\theta_A$ (°C) & Wind |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 18.2 | 15.68 | 18.2 | 8.0 | 4.09 | 71.0 | 0.20 | 2.27 | 1.5 | Fair |
| 16h06m | 1 | 17.9 | 16.24 | 21.0 | 8.1 | 4.10 | 71.1 | 0.13 | 1.80 | ... | 17.0 |
| 3.0m | 2.5 | 17.8 | 16.34 | 21.2 | 8.1 | 4.19 | 72.7 | 0.28 | 2.06 | | S-1 |
| **2** | 0 | 17.5 | 16.48 | 21.4 | 8.2 | 4.97 | 85.8 | <0.05 | 1.76 | >2.0 | Fair |
| 15h56m | 1.5 | 17.6 | 17.02 | 22.2 | 8.2 | 5.67 | 99.0 | 0.16 | 1.68 | 7d | 17.2 |
| 2.0m | | | | | | | | | | | S-1 |
| **3** | 0 | 17.8 | 16.41 | 21.4 | 8.2 | 5.02 | 87.2 | <0.05 | 1.89 | 1.5 | Fair |
| 15h50m | 1 | 17.7 | 16.58 | 21.5 | 8.2 | 4.97 | 86.7 | 0.06 | 1.58 | 7d | 17.0 |
| 3.5m | 3.0 | 17.4 | 16.99 | 22.2 | 8.2 | 5.16 | 89.7 | 0.15 | 1.50 | | S-1 |
| **4** | 0 | 17.8 | 16.86 | 21.5 | 8.2 | 5.76 | 100.7 | <0.05 | 1.80 | >2.0 | Fair |
| 15h42m | 1.5 | 17.7 | 17.03 | 22.1 | 8.2 | 5.77 | 100.9 | 0.06 | 1.61 | 7d | ... |
| 2.0m | | | | | | | | | | | S-1 |
| **5** | 0 | 17.6 | 16.58 | 21.5 | 8.2 | 5.75 | 99.8 | <0.05 | 1.66 | >2.0 | 17.7 |
| 15h18m | 1 | 17.9 | 16.85 | 21.8 | 8.2 | 5.73 | 100.4 | <0.05 | 1.64 | 7d | ... |
| 2.0m | 1.5 | 17.9 | 16.91 | 21.9 | 8.2 | 5.73 | 100.4 | <0.05 | 1.76 | | ... |
| **6** | 0 | 18.0 | 16.74 | 21.7 | 8.2 | 5.90 | 103.3 | <0.05 | 1.52 | 2.0 | ... |
| 15h03m | 1 | 17.6 | 16.89 | 22.0 | 8.2 | 5.64 | 98.3 | <0.05 | 1.52 | 7 | ... |
| 4.0m | 3.5 | 17.8 | 17.08 | 22.2 | 8.2 | 5.40 | 94.6 | <0.05 | 1.59 | | S-1 |
| **8** | 0 | 18.4 | 17.06 | 22.0 | 8.2 | 5.61 | 99.3 | <0.05 | 1.28 | 2.7 | Fair |
| 14h50m | 1 | 18.2 | 17.12 | 22.1 | 8.2 | 5.57 | 98.4 | <0.05 | 1.53 | 6 | 19.5 |
| 5.0m | 4.5 | 18.0 | 17.15 | 22.2 | 8.2 | 5.46 | 96.1 | <0.05 | 1.23 | | S-1 |
| **10** | 0 | 18.1 | 17.04 | 22.1 | 8.2 | 5.54 | 97.5 | <0.05 | 1.52 | 2.8 | Fair |
| 14h21m | 1 | 18.2 | 17.04 | 22.0 | 8.2 | 5.59 | 98.6 | <0.05 | 1.59 | 7 | 20.2 |
| 5.0m | 4.5 | 18.0 | 17.10 | 22.2 | 8.2 | 5.49 | 96.7 | <0.05 | 1.63 | | S-1 |
| **A** | 0 | 18.4 | 15.32 | 19.6 | 8.0 | 4.01 | 69.5 | 0.21 | 2.68 | 1.0 | Fair |
| 16h15m | 1 | 18.2 | 15.87 | 20.4 | 8.0 | 5.69 | 99.0 | 0.25 | 2.52 | ... | 18.5 |
| 2.5m | 2.0 | 17.9 | 16.17 | 20.9 | 8.0 | 3.74 | 64.9 | 0.23 | 2.09 | | S-1 |
| **B** | 0 | 18.7 | 17.20 | 22.1 | 8.2 | 5.50 | 98.0 | <0.05 | 1.26 | 3.1 | Fair |
| 14h37m | 1 | 18.7 | 17.21 | 22.1 | 8.2 | 5.53 | 98.6 | <0.05 | 1.33 | 6~7 | ... |
| 6.0m | 5.5 | 18.2 | 17.15 | 22.2 | 8.2 | 5.43 | 95.9 | 0.12 | 1.53 | | S-1 |
| **D** | 0 | 17.5 | 16.81 | 21.9 | ... | 5.61 | 97.4 | <0.05 | 1.37 | ... | ... |
| 15h28m | 0.5 | 17.7 | 16.82 | 21.8 | ... | 5.66 | 98.8 | 0.06 | 1.50 | 7d | ... |
| <1m | | | | | | | | | | | ... |

Table C-1. Flood (morning) : March 13, 1959

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr (m) | Weather & $\theta_A$ (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 10.0 | 16.32 | 22.6 | 8.2 | 6.45 | 96.7 | 0.06 | 1.51 | 2.0 | Cloudy |
| 11h39m | 1 | 10.2 | 17.38 | 24.1 | 8.2 | 6.92 | 105.8 | 0.12 | 1.30 | | 7.1 |
| 5.0m | 4.5 | 10.3 | 17.66 | 24.5 | 8.2 | 6.56 | 100.8 | 0.07 | 1.12 | | |
| **2** | 0 | 9.8 | 17.61 | 24.5 | 8.2 | 6.47 | 98.3 | 0.06 | 1.12 | 1.2 | Cloudy |
| 11h27m | 1 | 10.6 | 17.62 | 24.4 | 8.2 | 6.51 | 100.6 | 0.06 | 1.35 | | ... |
| 4.0m | 3.5 | 11.0 | 17.76 | 24.5 | 8.2 | 6.42 | 100.2 | 0.14 | 1.17 | | |
| **3** | 0 | 9.9 | 17.78 | 24.5 | 8.2 | 6.37 | 97.1 | <0.05 | 1.56 | 2.0 | Cloudy |
| 11h19m | 1 | 10.4 | 17.52 | 24.3 | 8.2 | 6.08 | 93.3 | <0.05 | 1.36 | | 6.6 |
| 3.5m | 3.0 | 10.8 | 17.70 | 24.5 | 8.2 | 6.56 | 101.9 | <0.05 | 1.22 | | |
| **4** | 0 | 9.4 | 17.60 | 24.6 | 8.2 | 7.24 | 109.0 | <0.05 | 1.38 | 2.5 | Cloudy |
| 11h06m | 1 | 10.0 | 17.57 | 24.4 | 8.2 | 6.73 | 102.6 | <0.05 | 1.28 | | ... |
| 3.5m | 3.0 | 10.1 | 17.60 | 24.5 | 8.3 | 6.64 | 100.9 | <0.05 | 1.25 | | |
| **5** | 0 | 9.3 | 17.40 | 24.3 | 8.2 | 6.49 | 97.4 | <0.05 | 1.33 | 2.0 | Cloudy |
| 10h54m | 1 | 9.7 | 17.39 | 24.2 | 8.2 | 6.55 | 99.1 | 0.09 | 1.39 | | 6.9 |
| 2.5m | 2.0 | 9.7 | 17.39 | 24.2 | 8.3 | 6.47 | 97.9 | 0.06 | 1.27 | | |
| **6** | 0 | 9.8 | 17.57 | 24.5 | 8.4 | 6.56 | 99.7 | <0.05 | 1.43 | 2.5 | Cloudy |
| 10h37m | 1 | 10.0 | 17.58 | 24.5 | 8.2 | 6.65 | 101.4 | <0.05 | 1.44 | | 6.2 |
| 5.0m | 4.5 | 10.4 | 17.85 | 24.8 | 8.3 | 6.48 | 99.8 | <0.05 | 1.39 | | |
| **7** | 0 | 9.8 | 17.70 | 24.6 | 8.2 | 6.64 | 101.1 | <0.05 | 1.28 | 3.1 | Cloudy |
| 10h22m | 1 | 10.5 | 17.68 | 24.5 | 8.3 | 6.56 | 101.2 | <0.05 | 1.38 | | 6.0 |
| 6.0m | 5.5 | 10.1 | 17.77 | 24.7 | 8.2 | 6.58 | 100.9 | <0.05 | 1.35 | | |
| **8** | 0 | 9.8 | 17.66 | 24.6 | 8.2 | 6.52 | 99.2 | <0.05 | 1.35 | 2.9 | Cloudy |
| 10h07m | 1 | 10.2 | 17.61 | 24.5 | 8.2 | 6.56 | 100.6 | <0.05 | 1.38 | | 5.2 |
| 6.0m | 5.5 | 10.2 | 17.82 | 24.8 | 8.2 | 6.49 | 99.7 | <0.05 | 1.31 | | |
| **9** | 0 | 9.8 | 17.71 | 24.7 | 8.2 | 6.59 | 100.3 | <0.05 | 1.22 | 2.9 | Cloudy |
| 09h57m | 1 | 10.0 | 17.68 | 24.6 | 8.2 | 6.62 | 101.1 | <0.05 | 1.35 | | ... |
| 5.0m | 4.5 | 10.3 | 17.88 | 24.8 | 8.2 | 6.38 | 98.3 | <0.05 | 1.44 | | |
| **10** | 0 | 9.8 | 17.76 | 24.7 | 8.2 | 6.60 | 100.5 | <0.05 | 1.47 | 2.5 | Cloudy |
| 09h38m | 1 | 10.3 | 17.73 | 24.6 | 8.2 | 7.72 | 118.8 | <0.05 | 1.57 | | 5.0 |
| 4.0m | 3.5 | 10.8 | 17.84 | 24.7 | 8.2 | 6.53 | 101.6 | 0.14 | 1.49 | | |

Table C-2. Ebb (afternoon) : March 13, 1959

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr (m) | Weather & $\theta_A$(°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 10.2 | 17.45 | 24.2 | 8.2 | 6.54 | 100.0 | <0.05 | 1.30 | 2.2 | Cloudy |
| 15h16m | 1 | 10.4 | 17.39 | 24.1 | 8.2 | 6.54 | 100.3 | <0.05 | 1.12 | | 7.6 |
| 5.0m | 4.5 | 10.4 | 17.69 | 24.5 | 8.2 | 6.59 | 101.4 | <0.05 | 1.20 | | |
| **2** | 0 | 9.8 | 17.73 | 24.7 | 8.2 | 6.74 | 102.6 | <0.05 | 1.03 | 2.3 | Cloudy |
| 15h22m | 1 | 10.3 | 17.73 | 24.6 | 8.2 | 6.76 | 103.8 | <0.05 | 0.99 | | 8.6 |
| 4.0m | 3.5 | 10.8 | 17.81 | 24.6 | 8.2 | 6.71 | 104.4 | <0.05 | 1.07 | | |
| **3** | 0 | 10.3 | 17.74 | 24.6 | 8.2 | 6.68 | 102.8 | <0.05 | 0.96 | 2.8 | Cloudy |
| 15h35m | 1 | 10.4 | 17.74 | 24.6 | 8.2 | 6.68 | 102.9 | <0.05 | 1.09 | | 8.2 |
| 4.0m | 3.5 | 10.6 | 17.75 | 24.6 | 8.2 | 6.55 | 101.4 | <0.05 | 1.04 | | |
| **4** | 0 | 9.9 | 17.60 | 24.5 | 8.2 | 6.78 | 103.2 | <0.05 | 1.15 | 2.5 | Cloudy |
| 15h44m | 1 | 10.3 | 17.63 | 24.5 | 8.2 | 6.57 | 100.9 | <0.05 | 1.31 | | 7.8 |
| 3.0m | 2.5 | 10.5 | 17.65 | 24.5 | 8.2 | 6.75 | 104.0 | <0.05 | 1.12 | | |
| **5** | 0 | 9.8 | 17.55 | 24.4 | 8.2 | 6.83 | 103.6 | <0.05 | 1.27 | 2.5 | Cloudy |
| 15h51m | 1 | 10.2 | 17.57 | 24.4 | 8.2 | 6.96 | 106.6 | <0.05 | 1.27 | | 7.9 |
| 3.0m | 2.5 | 10.4 | 17.57 | 24.4 | 8.2 | 6.83 | 104.9 | 0.16 | 1.54 | | |
| **6** | 0 | 9.7 | 17.56 | 24.5 | 8.2 | 6.93 | 105.0 | <0.05 | 1.22 | 2.1 | Cloudy |
| 16h02m | 1 | 10.0 | 17.55 | 24.4 | 8.2 | 6.77 | 103.2 | <0.05 | 1.28 | | 8.3 |
| 4.8m | 4.5 | 10.4 | 17.57 | 24.4 | 8.2 | 6.82 | 104.8 | 0.06 | 1.52 | | |
| **7** | 0 | 9.7 | 17.79 | 24.8 | 8.2 | 7.34 | 111.6 | <0.05 | 1.28 | 3.0 | Cloudy |
| 16h12m | 1 | 10.0 | 17.78 | 24.7 | 8.2 | 6.69 | 102.3 | <0.05 | 1.14 | | 7.6 |
| 5.5m | 5.0 | 10.4 | 17.80 | 24.8 | 8.2 | 6.64 | 102.3 | <0.05 | 1.43 | | |
| **8** | 0 | 10.1 | 17.77 | 24.7 | 8.2 | 6.66 | 102.1 | <0.05 | 1.28 | 2.9 | Cloudy |
| 16h24m | 1 | 10.2 | 17.78 | 24.7 | 8.2 | 6.65 | 102.2 | <0.05 | 1.12 | | 7.6 |
| 5.5m | 5.0 | 10.5 | 17.83 | 24.7 | 8.2 | 6.68 | 103.2 | <0.05 | 1.15 | | |
| **9** | 0 | 10.1 | 17.77 | 24.7 | 8.2 | 6.67 | 102.3 | <0.05 | 1.43 | 2.9 | Cloudy |
| 16h32m | 1 | 10.3 | 17.76 | 24.6 | 8.2 | 6.85 | 105.4 | <0.05 | 1.25 | | 7.6 |
| 4.5m | 4.0 | 10.5 | 17.83 | 24.7 | 8.3 | 6.69 | 103.4 | <0.05 | 1.27 | | |
| **10** | 0 | 10.0 | 17.80 | 24.8 | 8.2 | 6.72 | 102.8 | <0.05 | 1.28 | 2.9 | Cloudy |
| 16h38m | 1 | 10.2 | 17.81 | 24.7 | 8.2 | 6.80 | 104.5 | <0.05 | 1.12 | | 7.6 |
| 4.5m | 4.0 | 10.9 | 17.82 | 24.6 | 8.2 | 6.68 | 104.0 | 0.09 | 1.51 | | |

Table D–1. Ebb (morning) : July 1, 1959

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr(m) & $C_F$ | Weather & $\theta_A$ (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0 | 27.1 | 17.06 | 19.6 | 8.1 | 4.16 | 85.4 | 0.05 | 1.73 | 2.7 | Cloudy |
| 09h03m | 1 | 26.3 | 17.40 | 20.3 | 8.1 | 4.81 | 97.8 | 0.02 | 1.17 | 6 | 24.5 |
| 3.0m | 2.5 | 26.2 | 17.38 | 20.3 | 8.1 | 4.52 | 91.9 | 0.13 | 1.47 | | |
| **2** | 0 | 26.1 | 17.40 | 20.4 | 8.2 | 4.87 | 98.6 | 0.01 | 0.66 | 3.4 | Cloudy |
| 09h40m | 1 | 25.9 | 17.33 | 20.3 | 8.3 | <4.89 | <98.6 | 0.01 | 0.82 | … | … |
| 3.5m | 3.0 | 25.1 | 17.51 | 20.8 | 8.1 | 4.18 | 83.3 | 0.10 | 0.88 | | |
| **3** | 0 | 26.0 | 17.33 | 20.3 | 8.1 | 5.18 | 104.6 | 0.02 | 0.82 | 3.5 | Cloudy |
| 09h47m | 1 | 25.8 | 17.46 | 20.5 | 8.2 | 5.24 | 105.4 | 0.02 | 0.61 | 5~6 | 25.0 |
| 4.0m | 3.5 | 25.7 | 17.43 | 20.5 | 8.2 | 5.18 | 104.2 | 0.04 | 0.80 | | |
| **4** | 0 | 26.1 | 16.92 | 19.7 | 8.2 | 5.14 | 103.4 | 0.01 | 0.62 | 3.6 | Cloudy |
| 09h57m | 1 | 25.9 | 17.35 | 20.3 | 8.1 | 5.30 | 106.9 | 0.01 | 0.64 | 5~6 | 25.1 |
| 4.0m | 3.5 | 25.3 | 17.42 | 20.6 | 8.1 | 4.67 | 93.2 | 0.03 | 0.64 | | |
| **5** | 0 | 26.7 | 16.85 | 19.4 | 8.2 | 5.28 | 107.3 | 0.04 | 1.04 | 2.5 | Cloudy |
| 10h10m | 1 | 26.3 | 16.99 | 19.7 | 8.2 | 5.34 | 107.9 | 0.04 | 1.01 | 7 | 25.0 |
| 3.0m | 2.5 | 25.9 | 17.42 | 20.4 | 8.1 | 3.79 | 76.4 | 0.04 | 0.70 | | |
| **6** | 0 | 26.4 | 16.23 | 18.7 | 8.1 | 5.14 | 103.2 | 0.10 | 1.17 | 3.3 | Fair |
| 10h30m | 1 | 25.5 | 17.36 | 20.5 | 8.2 | 5.36 | 107.2 | 0.02 | 0.61 | 7 | 25.1 |
| 4.5m | 4.0 | 24.8 | 17.46 | 20.8 | 8.2 | 4.73 | 93.7 | 0.04 | 0.56 | | |
| **7** | 0 | 26.3 | 17.16 | 20.0 | 8.2 | 5.40 | 109.3 | 0.02 | 0.74 | 3.5 | Fair |
| 10h41m | 1 | 26.2 | 17.32 | 20.2 | 8.3 | 5.47 | 110.7 | 0.03 | 0.94 | 5~6 | 25.4 |
| 5.5m | 5.0 | 24.0 | 17.50 | 21.1 | 8.2 | 4.29 | 83.8 | 0.04 | 0.96 | | |
| **8** | 0 | 26.1 | 17.37 | 20.3 | 8.2 | 5.26 | 106.5 | 0.02 | 0.59 | 3.9 | Fair |
| 10h52m | 1 | 25.9 | 17.47 | 20.5 | 8.3 | 4.60 | 92.7 | 0.02 | 0.56 | 5 | 25.4 |
| 5.0m | 4.5 | 24.1 | 17.56 | 21.2 | 8.2 | 4.14 | 81.2 | 0.18 | 1.25 | | |
| **9** | 0 | 26.4 | 17.47 | 20.4 | 8.2 | 5.24 | 106.7 | 0.02 | 0.69 | 3.7 | Cloudy |
| 11h01m | 1 | 26.2 | 17.43 | 20.4 | 8.2 | 5.24 | 106.3 | 0.01 | 0.58 | 5 | 25.8 |
| 4.5m | 4.0 | 25.3 | 17.43 | 20.6 | 8.3 | 5.22 | 104.2 | 0.02 | 0.66 | | |
| **10** | 0 | 26.2 | 17.44 | 20.4 | 8.3 | 5.36 | 108.7 | 0.01 | 0.64 | 3.6 | Fair |
| 11h10m | 1 | 26.0 | 17.43 | 20.4 | 8.2 | 5.26 | 106.3 | 0.02 | 1.20 | 5 | 26.0 |
| 4.0m | 3.5 | 25.3 | 17.46 | 20.7 | 8.2 | 5.28 | 105.6 | 0.03 | 0.90 | | |
| **A** | 0 | 26.8 | 13.82 | 15.3 | 7.5 | 1.21 | 23.8 | 0.64 | 3.89 | 1.9 | Cloudy |
| 09h25m | 1 | 26.9 | 17.07 | 19.7 | 8.1 | 3.91 | 79.1 | 0.03 | 1.17 | 7 | … |
| 7.0m | 6.5 | 26.2 | 17.37 | 20.3 | 8.1 | 4.44 | 90.1 | 0.07 | 1.04 | | |
| **B** | 0 | 26.4 | 17.47 | 20.4 | 8.3 | 4.74 | 96.5 | 0.03 | 0.69 | 4.4 | Fair |
| 11h20m | 1 | 25.9 | 17.47 | 20.5 | 8.3 | 4.54 | 91.5 | trace | 0.69 | 5 | 25.8 |
| 6.0m | 5.5 | 24.3 | 17.47 | 20.9 | 8.3 | 5.79 | 113.5 | 0.01 | 0.61 | | |

Table D-2. Early flood (afternoon) : July 1, 1959

| Station, Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Fe (ppm) | COD (ppm) | Tr(m) & $C_F$ | Weather & $\theta_A$ (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1**<br>15h45m<br>0.8m | 0 | 28.9 | 6.85 | 5.3 | 7.3 | 3.40 | 64.3 | 0.50 | 5.30 | 1.0<br>br | Cloudy<br>28.3 |
| **2**<br>15h32m<br>2.0m | 0<br>1<br>1.5 | 27.4<br>27.2<br>27.0 | 17.35<br>17.31<br>17.33 | 19.9<br>19.9<br>20.0 | 8.4<br>8.3<br>8.2 | 6.26<br>5.94<br>5.32 | 129.6<br>122.5<br>109.2 | 0.04<br>0.01<br>0.03 | 0.96<br>0.93<br>0.80 | 2.0<br>7 | Cloudy<br>27.1 |
| **3**<br>15h27m<br>2.5m | 0<br>1<br>2.0 | 27.4<br>27.1<br>26.2 | 17.22<br>17.36<br>17.42 | 19.7<br>20.0<br>20.3 | 8.3<br>8.2<br>8.2 | 6.24<br>6.10<br>5.02 | 128.9<br>125.5<br>101.8 | 0.03<br>0.03<br>0.02 | 1.04<br>0.99<br>0.90 | 2.2<br>7 | Cloudy<br>26.7 |
| **4**<br>15h15m<br>2.5m | 0<br>1<br>2.0 | 27.4<br>27.4<br>27.0 | 17.17<br>17.10<br>17.41 | 19.6<br>19.5<br>20.1 | 8.4<br>8.3<br>8.3 | 7.19<br>7.17<br>5.59 | 148.6<br>147.8<br>115.0 | 0.05<br>0.02<br>0.02 | 1.55<br>1.49<br>6.02 | 2.3<br>8d | Cloudy<br>27.1 |
| **5**<br>15h04m<br>1.5m | 0<br>1 | 27.2<br>27.2 | 17.11<br>17.10 | 19.6<br>19.6 | 8.4<br>8.3 | 6.57<br>6.71 | 135.2<br>137.8 | 0.04<br>0.06 | 1.46<br>1.54 | 1.5<br>8d | Cloudy<br>27.1 |
| **6**<br>14h53m<br>3.5m | 0<br>1<br>3.0 | 26.3<br>26.3<br>26.0 | 17.38<br>17.32<br>17.38 | 20.3<br>20.2<br>20.4 | 8.4<br>8.3<br>8.1 | 5.59<br>5.55<br>5.36 | 113.6<br>112.6<br>108.3 | 0.02<br>0.01<br>0.02 | 1.46<br>1.20<br>0.88 | 3.5<br>5 | Cloudy<br>26.2 |
| **7**<br>14h40m<br>5.0m | 0<br>1<br>4.5 | 26.2<br>26.2<br>24.8 | 17.44<br>17.46<br>17.50 | 20.4<br>20.4<br>20.9 | 8.2<br>8.1<br>8.2 | 5.47<br>5.49<br>5.18 | 111.0<br>111.4<br>102.6 | trace<br>trace<br>0.04 | 0.85<br>0.90<br>0.85 | 3.8<br>5 | Cloudy<br>26.3 |
| **8**<br>14h27m<br>4.5m | 0<br>1<br>4.0 | 26.4<br>26.5<br>24.4 | 17.49<br>17.44<br>17.52 | 20.4<br>20.3<br>21.0 | 8.2<br>8.2<br>8.3 | 5.51<br>5.42<br>5.34 | 112.2<br>110.4<br>105.1 | 0.02<br>0.01<br>0.05 | 0.91<br>0.69<br>0.82 | 3.6<br>7 | Cloudy<br>26.7 |
| **9**<br>14h10m<br>4.0m | 0<br>1<br>3.5 | 26.7<br>26.6<br>25.3 | 17.45<br>17.44<br>17.52 | 20.2<br>20.2<br>20.8 | 8.2<br>8.2<br>8.3 | 5.40<br>5.43<br>5.24 | 110.7<br>110.8<br>104.8 | 0.02<br>0.01<br>0.04 | 0.72<br>0.70<br>0.85 | 3.3<br>5~6 | Cloudy<br>27.6 |
| **10**<br>14h00m<br>3.5m | 0<br>1<br>3.0 | 27.4<br>26.6<br>26.0 | 17.40<br>17.45<br>17.53 | 19.9<br>20.3<br>20.6 | 8.3<br>8.3<br>8.2 | 5.51<br>5.43<br>4.91 | 114.1<br>112.4<br>99.4 | 0.02<br>0.02<br>0.04 | 1.49<br>1.01<br>0.99 | 2.7<br>6 | Cloudy<br>27.8 |
| **A**<br>15h55m<br>6.0m | 0<br>1<br>5.5 | 28.7<br>27.7<br>26.8 | 5.38<br>14.97<br>17.13 | 3.4<br>15.6<br>19.8 | 7.4<br>8.0<br>8.2 | 3.95<br>2.29<br>3.64 | 73.4<br>46.4<br>74.3 | 0.55<br>0.17<br>0.11 | 5.95<br>1.84<br>0.90 | 0.9<br>br | Cloudy<br>27.8 |
| **B**<br>16h12m<br>5.0m | 0<br>1<br>4.5 | 26.0<br>26.4<br>24.2 | 17.43<br>17.38<br>17.50 | 20.4<br>20.2<br>21.1 | 8.3<br>8.1<br>8.1 | 5.38<br>5.36<br>5.65 | 108.7<br>108.9<br>110.8 | trace<br>0.01<br>0.02 | 0.56<br>0.58<br>0.80 | 4.1<br>5 | Cloudy<br>26.8 |

Table E. Data of "12-Hour Observation" at St. T on July 10, 1960.

| Time & Sounding | Z (m) | $\theta_W$ (°C) | Cl (‰) | $\sigma_t$ | pH | $O_2$ (cc/L) | $O_2$ (%) | Tr(m) & $C_F$ | Ex. coef. 0–1m | Surface Current (cm/sec) | Weather & $\theta_A$(°C) | Wind |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5h30m | 0 | 25.5 | 4.54 | 3.2 | 7.2 | 3.11 | 54.2 | 0.8 | 1.99 | ESE | Fair | 0 |
| 2.2m | 1 | 24.9 | 16.21 | 19.1 | 8.0 | 4.11 | 80.3 | 10～11 | | 28 | 25.7 | |
| 6h30m | 0 | 24.9 | 6.68 | 6.2 | 7.2 | 3.03 | 53.5 | 0.8 | 2.67 | ESE | Fair | NNW–1 |
| 2.0m | 1 | 24.6 | 15.17 | 17.8 | 8.1 | 4.19 | 80.6 | 10～11 | | 14 | 27.5 | |
| 7h30m | 0 | 25.7 | 9.49 | 9.8 | 8.1 | (4.60) | (84.7) | 1.1 | 1.31 | WNW | Fair | WSW–1 |
| 2.3m | 1 | 25.2 | 15.02 | 17.4 | 8.1 | 5.01 | 97.1 | 10 | | 7 | … | |
| 8h30m | 0 | 26.2 | 10.06 | 10.4 | 8.1 | 4.81 | 90.0 | 1.5 | 1.49 | NE | Fair | WSW–1 |
| 2.9m | 2 | 25.1 | 15.69 | 18.3 | 8.1 | 5.41 | 105.5 | 10 | | … | 27.8 | |
| 9h45m | 0 | 25.7 | 14.16 | 16.1 | 8.1 | 6.00 | 116.1 | 1.5 | 1.33 | SW | Rain | NNW–1 |
| 3.8m | 2 | 25.2 | 15.52 | 18.1 | 8.1 | 5.76 | 112.3 | 10 | | 6 | 27.4 | |
| 10h30m | 0 | 25.9 | 12.17 | 13.3 | 8.1 | … | … | 1.8 | 1.40 | SSW | Rain | NW–1 |
| 4.3m | 2 | 25.0 | 16.05 | 18.9 | 8.1 | … | … | 10 | | 6 | 27.5 | |
| 11h30m | 0 | 25.7 | 14.95 | 17.1 | 8.1 | 6.37 | 124.2 | 1.5 | 1.30 | SSE | Cloudy | NW–1 |
| 4.6m | 2 | 24.9 | 16.21 | 19.1 | 8.1 | 5.46 | 106.6 | 10 | | 7 | 28.6 | |
| 12h30m | 0 | 25.5 | 15.47 | 17.9 | 8.1 | 6.40 | 125.5 | 1.7 | 0.97 | ESE | Fair | SSW–1 |
| 4.5m | 2 | 24.9 | 16.25 | 19.1 | 8.1 | … | … | 10 | | 16 | 28.5 | |
| 13h30m | 0 | 25.2 | 13.77 | 15.7 | 8.1 | … | … | 1.9 | 1.05 | SE | … | WNW–1 |
| 3.9m | 2 | 24.5 | 16.9 | … | 8.1 | … | … | 9 | | 31 | 26.6 | |
| 14h30m | 0 | 26.2 | 14.27 | 16.1 | 8.1 | 5.46 | 106.8 | 1.8 | 1.38 | ESE | Cloudy | W–1 |
| 3.1m | 2 | 24.1 | 16.95 | 20.3 | 8.1 | 4.45 | 86.6 | 9 | | 31 | 28.0 | |
| 15h30m | 0 | 25.7 | 15.07 | 17.3 | 8.1 | … | … | 1.7 | 1.72 | … | Cloudy | WNW–1 |
| 2.4m | 2 | 24.3 | 17.24 | 20.7 | 8.1 | … | … | 9 | | 38 | 27.8 | |
| 16h30m | 0 | 27.3 | 4.51 | 2.6 | <7.2 | 4.33 | 77.9 | 0.7 | 2.78 | SE | … | NW |
| 1.8m | 1 | 25.4 | 16.05 | 18.7 | 8.1 | 4.14 | 81.7 | 9 | | 45 | 28.9 | |
| 17h30m | 0 | 27.4 | 5.44 | 3.8 | <7.2 | 4.18 | 74.8 | 0.5 | 3.10 | SSE | … | SW–1 |
| 1.3m | 1 | 25.4 | 15.01 | 17.3 | 8.1 | 3.64 | 70.8 | 10 | | 10 | 28.8 | |

Table F. Data of "Land Drainage Survey" on July 11, 1960.

| Station | | K1 | K2 | K3 | K4 | K5 | K6 | K7 | K8 |
|---|---|---|---|---|---|---|---|---|---|
| Time | | 11h05m | 11h18m | 11h28m | 11h39m | 11h55m | 12h15m | 12h33m | 12h45m |
| $\theta_A$ (°C) | | ... | ... | 26.4 | ... | ... | ... | ... | ... |
| Wind | | S-2 | ... | ... | ... | ... | ... | ... | ... |
| Sounding (m) | | 5.2 | 7.3 | 7.9 | 8.1 | 10.5 | 12.0 | 6.0 | 6.5 |
| Tr (m) | | 1.5 | 1.9 | 3.0 | 3.0 | 2.1 | 2.2 | 1.6 | 1.3 |
| $\theta_W$ (°C) | 0m | 28.1 | 28.4 | 25.3 | 25.0 | 24.6 | 24.3 | 26.6 | 27.5 |
| | 1m | 27.5 | 27.3 | 24.7 | 24.3 | 24.6 | 24.2 | 26.0 | 27.4 |
| | 2m | 24.6 | 24.1 | 23.4 | 24.0 | 22.9 | 23.9 | 25.7 | 26.5 |
| | 3m | 23.7 | 23.4 | 23.0 | 23.1 | 22.8 | 23.6 | 25.4 | 24.2 |
| | 4m | 23.5 | 23.1 | 23.0 | 23.0 | 22.8 | 23.2 | 24.8 | 23.7 |
| | 5m | 23.5 | 22.9 | 22.9 | 22.9 | 22.7 | 23.0 | 24.2 | 23.6 |
| | 6m | | 22.9 | 22.9 | 22.9 | 22.7 | 23.0 | 23.3 | 23.6 |
| | 7m | | 22.9 | 22.8 | 22.8 | 22.7 | 22.9 | | |
| | 8m | | | | 24.8 | 22.7 | 22.9 | | |
| | 9m | | | | | 22.7 | 22.9 | | |
| | 10m | | | | | 22.7 | 22.9 | | |
| | 11m | | | | | | 22.9 | | |
| | 12m | | | | | | 22.9 | | |
| Cl (‰) | 0m | 13.18 | 14.34 | 16.63 | 16.85 | 17.00 | 16.88 | 14.5 | 14.3 |
| | 5m | 17.33 | ... | ... | ... | 17.48 | ... | 17.4 | 17.32 |
| | 6m | | 17.48 | 17.48 | 17.48 | ... | ... | ... | ... |
| | 7m | | ... | ... | ... | ... | 17.46 | | ... |

# On Two New Eledonid Octopods from The Antarctic Sea

Iwao TAKI

*Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama*

(Pls. 1—3; Text-figs. 1—16; Tables 1—3)

During her observatory cruise of the research vessel "Umitaka-maru" attached to the Tokyo University of Fisheries to the Antarctic Sea in 1957, three specimens of Eledonid octopods were obtained, together with some benthic invertebrates, with the beam-trawl by Prof. Jirô SENÔ, of the same University, somewhere near the "Shôwa Base" of the Antarctic Observatory Party of Japan. They were handed over to me for specific identification in 1960. It was revealed that the largest specimen out of these three represents a new subfamily, new genus and species, namely, Megaleledoninae, *Megaleledone senoi*, and two smaller specimens, a new species, *Pareledone umitakae*. The descriptions of these two species, accompanied by some morphological and zoogeographical considerations, are embodied in the present report.

I wish to express my cordial thanks to Prof. SENÔ by whose kindness I could have an opportunity of observing so rare specimens, hitherto inaccessible in Japan. Dr. Shirô MURAKAMI, Saikai Regional Fisheries Research Station, Nagasaki, kindly tried to identify the broken pieces of Ophiuroid animals contained in the stomach of *Megaleledone senoi*, to whom I am much obliged. My thanks are also due to Dr. Katura OYAMA, of the Geological Survey of Japan, Tokyo, who was kind enough to lend me some important literatures. In both 1960 and 1961 my study on these specimens has been assisted by a grant of Scientific Testing Research from the Ministry of Education, for which I am very grateful.

## DESCRIPTION OF THE SPECIES

### Family Octopodidae
### Subfamily Megaleledoninae, new subfamily
### Genus *Megaleledone* TAKI, new genus

An eledonid of a great size; mantle wider than long, ink-sac small, crop absent; rhachidian tooth of radula symmetrical, unicuspid. Ovarian eggs large.
Type-species: *Megaleledone senoi*, TAKI, new species. (*megalē*, Gr. large, great, big.)

### *Megaleledone senoi*[1] TAKI, new species
(Pls. 1—2; Text-figs. 1—8, 16)

*General appearance:* Body generally plump, rather soft, surface smooth without

1) It is my pleasure that I name this species in honour of Prof. SENÔ who collected this valuable specimen.

any dermal sculpture, though irregularly wrinkled due to the action of the preservative. Integument is thick and flabby, quite loose and easily dislocated here and there to form wrinkles, though muscular tissue rather firm. Light purplish brown (light 'Hydrangea red') in colour, paler on the ventral side of body.

One specimen, female.

Locality: 67° 51.5′ S., 33° 13.5′ E. Depth: 630–680 m.

Date: 7. Feb., 1957. 16.01–17.50 (Hour).

Gear: Beam-trawl. R.V. "Umitaka-maru."

*Measurements:* (cf. ROBSON 1929; PICKFORD 1945)

Total length: 460 mm.

Weight: 2860 g (measured just after capture)

Mantle length, dorsal 135 mm., ventral 102 mm.;

Mantle width 143 mm, index 106;

width of head 89 mm, index 66; sucker index 11; funnel length 25 mm., width of aperture 10 mm.

Arm:

| No. | Left | | | Right | | |
|---|---|---|---|---|---|---|
| | length mm. | diam. largest sucker mm. | no. sucker | length mm. | diam. largest sucker mm. | no. sucker |
| 1 | 281 | 14 | 62 | 278 | 14 | 61 |
| 2 | 255+a | 14 | 35+a | 292 | 14 | 63 |
| 3 | 285 | 14 | 61 | 280 | 15 | 62 |
| 4 | 280 | 14 | 61 | 295 | 15 | 63 |

Web-depth:

| | | Left mm. | Right mm. |
|---|---|---|---|
| Sector | A | 108 | |
| | B | 130 | 120 |
| | C | 125 | 120 |
| | D | 116 | 102 |
| | E | 90 | |

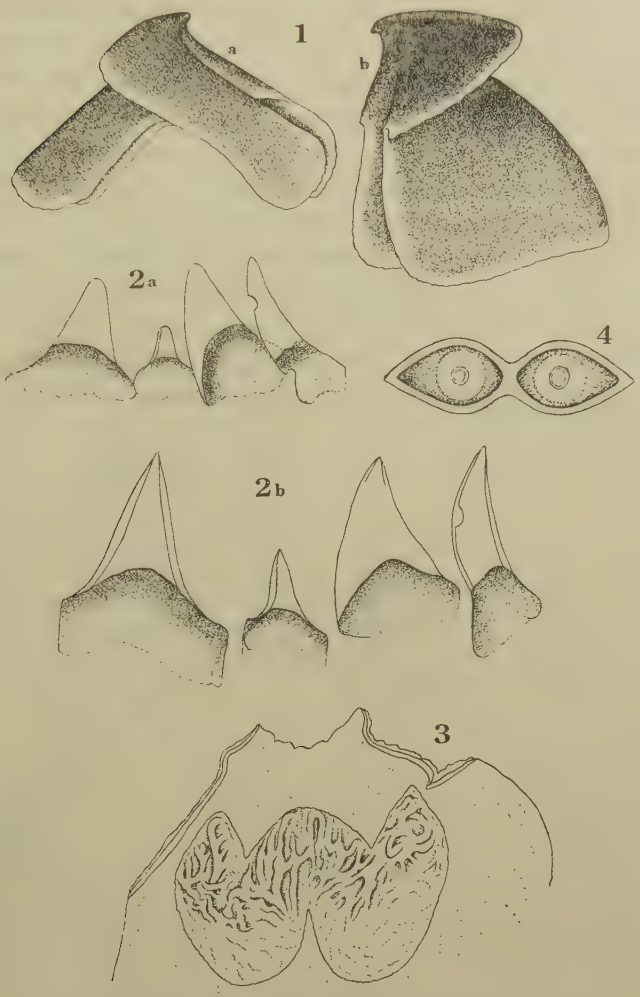
Gill: No. primary leaflet

| Left | | Right | |
|---|---|---|---|
| inner | outer | inner | outer |
| 11 | 11 | 12 | 11 |

*Mantle and head:* Mantle is transversely ellipsoid in outline (width index 106). This is not due to the post-mortem deformation of the specimen in the preservative, as actually witnessed by Prof. SENÔ who collected it. The mantle opening is very wide. Head is narrower than mantle; no ocular cirrus observed.

*Arm:* Generally squat and big, their length subequal, namely, 4. 2. 3. 1, the longest arm is 64% in the total length and mantle-length is 46% in the length of longest arm. Web is well developed, its order is B. C. D. A. E., and the depth of the deepest sector is 44% of the longest arm.

*Sucker:* Uniserial, relatively large, its greatest diameter is 11% in the mantle-length, and its number is fairly numerous.




Text-figs. 1–4. *Megaleledone senoi* TAKI, n. g. et sp.
1 a, lower mandible; b upper mandible, × ca. 1;
2. Radula; 2a, 4th row: 2b, 29th row. × ca. 50.
3. Funnel-organ, cut open. × ca. $\frac{3}{4}$.
4. Statocyst, cut open, ventral view. × ca. 1.1.

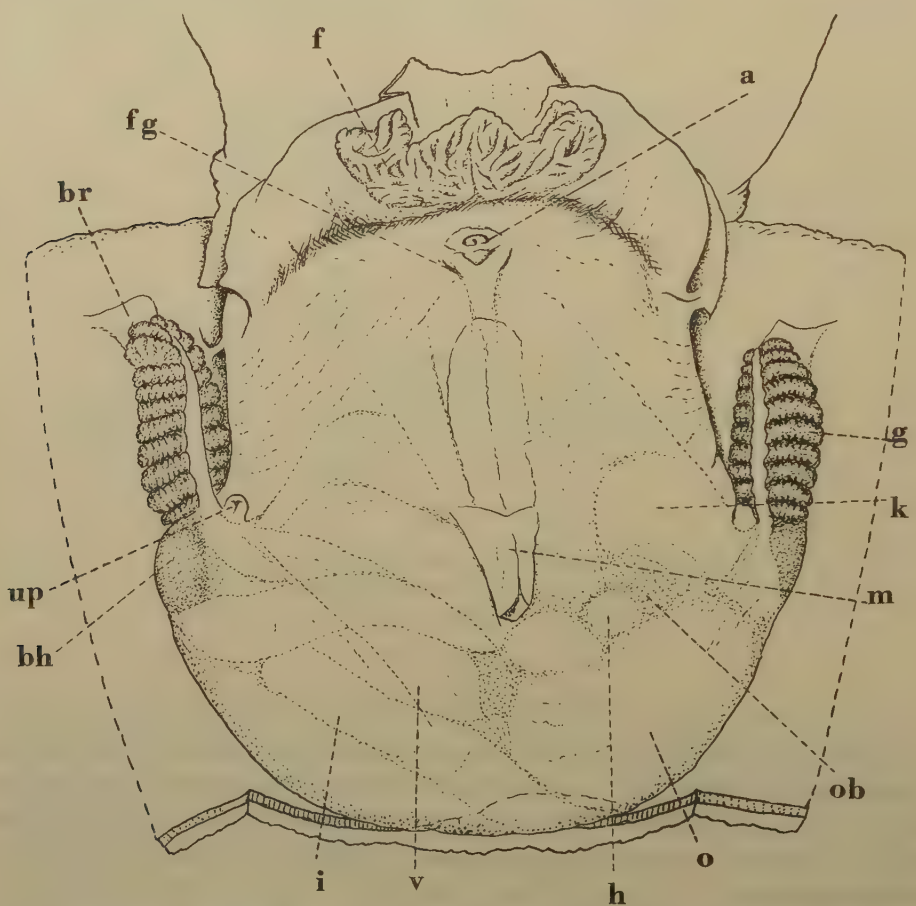
*Funnel* (Text-fig. 3)*:* Wide and squat; funnel-organ W-shaped, its limb is exceedingly wide, so that the two anterior and posterior sinuations are very shallow. The organ is quite shrunken to produce many deep wrinkles on the anterior half area, but quite low and the boundary between it and the neighbouring integument is rather indistinct.

*Buccal bulb:* Roughly spherical, though somewhat elongated dorso-ventrally, measuring ca. 40 mm wide, 45 mm high and 40 mm long. Mandibles (Text-fig. 1) are well developed, both rostra quite stout, with a blunt apex and deep black in colour. Ala of lower mandible wide and long, 33 × 16 mm on each side, jugular

lamina 31 mm long and 12 mm wide. Rostrum of upper mandible incurved and its apex rounded owing to abrasion, frontal lamina 20 mm long and 24 mm wide on each side, palatine lamina 38 mm high and 29 mm long at the base.

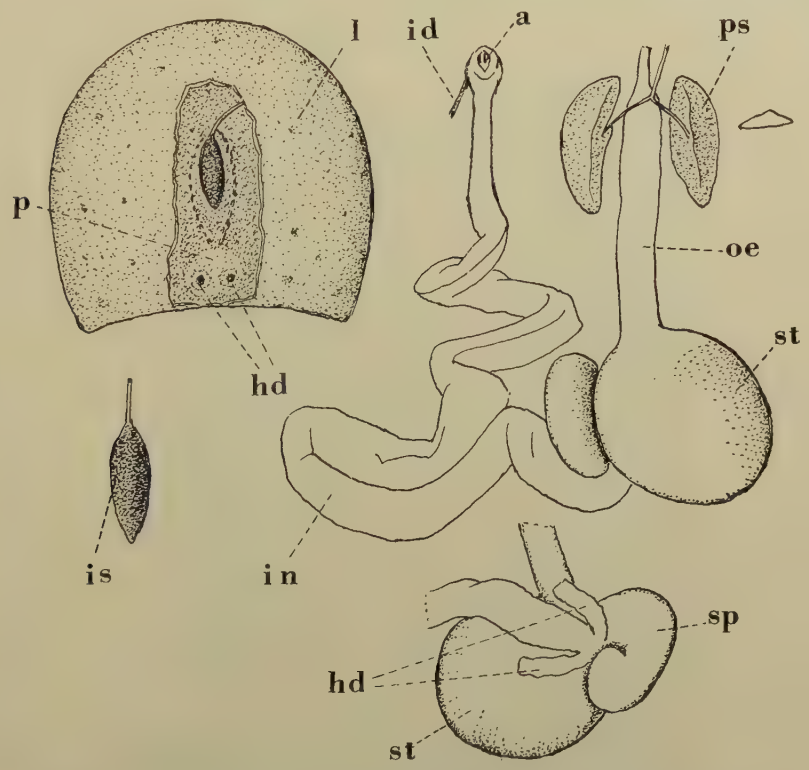
*Radula* (Text-figs. 2a, 2b): Quite large; when extended it measures 9 mm in width. Anterior part of the radula is rather small and tip of each cusp of teeth blunt, probably due to the abrasion by rasping action. Teeth transparent, very faintly brown in colour, the thickened base dark brown.

The 4th row (2a): Rhachidian rather low, with a blunt cusp and moderately wide base; cusp of first lateral rather wide and blunt, with a thin and narrow blade on its inner margin; second lateral quite large in comparison with the other teeth, with a blunt cusp, its inner margin faintly arcuated; third lateral rather narrow and long, gently arcuated with a blunt apex, a smal elliptical notch at the middle of the inner margin of the cusp as well as at the inside of the base.




Text-fig. 5. *Megaleledone senoi* TAKI, n. g. et sp. Ventral view of the viscera, mantle and funnel cut open, ca. half nat. size.
a, anus; bh, branchial heart; br, branchial gland; f, funnel-organ; fg, female genital opening; g, gill; h, arterial heart; i, intestine; k, kidney; m, mantle septum; o, ovary; ob, left oviducal ball; up, urinary papilla; v, right vagina.

The 29th row (2b): Teeth generally larger than those of the anterior part. Rhachidian thin and long, with a sharply pointed cusp, and a thin blade on both sides, base wide, roughly pentagonal; first lateral long and pointed, a thin blade at its inner margin, base wide, with a small and low process at its inner margin, which does not develop into an ectocone anywhere in the radula; second lateral wide and rather pointed, blade very narrow and short, base wide; third lateral wide and long, a thin blade on both sides, with a small notch at its inner margin (this notch can be observed throughout the right third lateral up to 48th row, though no such peculiar incision exists on the left side.) No marginal plate.



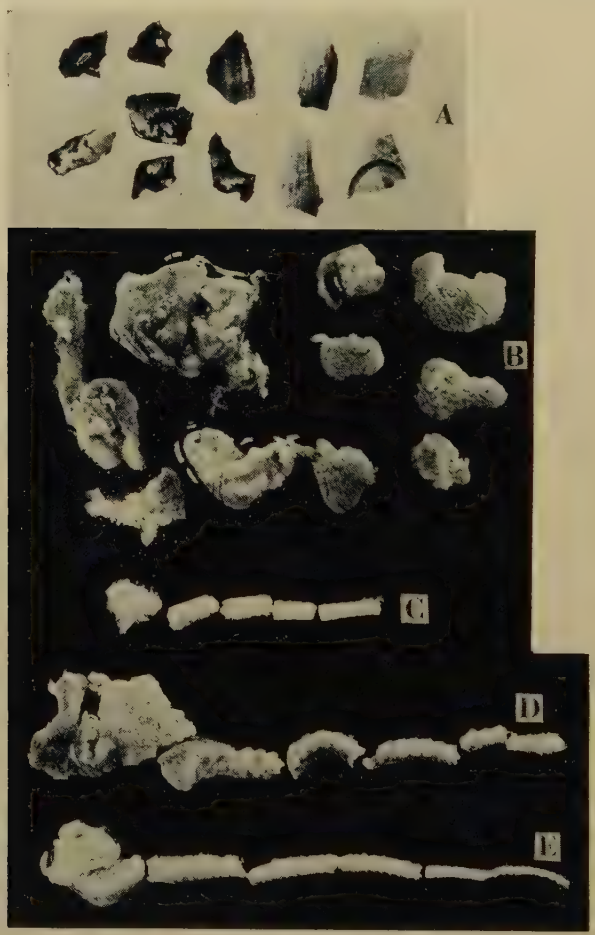

Text-fig. 6. *Megaleledone senoi* TAKI, n. g. et sp. Alimentary canal, ×ca. 3/5. a, anus; h, hepatic duct; id, ink-duct; in, intestine; is, ink-sac; l, liver; broken line at the centre shows the outline of ink-sac imbedded in the liver; oe, oesophagus; p, pancreas; ps, posterior salivary gland, and its cross section; st, stomach; sp, spiral caecum.

*Oesophagus* (Text-fig. 6, oe): anterior part 6 mm wide and 18 mm long, but posterior part widened, 15 mm long, nearly cylindrical, without any particular diverticle which can be discriminated as a crop.

*Posterior salivary gland* (ps) ellipsoid and flat, 33 mm long, 15 mm wide and 4 mm thick, discoloured in ashy brown, somewhat resembling the colour of the liver, perhaps due to the post-mortem oxidation of the tissue. Such discolouration, though in a weak degree, is usually met with in *Octopus vulgare*.

*Liver* (l) massive, of an enormous size, 82 mm wide and 70 mm long, roughly transversely oblong, completely covered with a thin muscular envelope, on which dark dots (1–5 mm in diameter) are sparsely scattered all over the surface; ink-sac (is) embedded at about the centre of the ventral side of the liver; pancreas (p) 20 mm wide and 15 mm long, shallowly bilobed at the posterior side, thin ashy buff in colour; through the tissue of pancreas two hepatic ducts (hd) pour into intestine at the junction between it and the spiral caecum (sp).

*Ink-sac* (is): A part of its surface appears as a fusiform, $7 \times 21$ mm in size, on the ventral surface of the liver, but its bulk is buried therein, the total sac measuring $13 \times 27$ mm in size, which is fusiform tapering to both the anterior and posterior ends, connected with a slender stalk (id) to the dorsal side of the anus.




Text-fig. 7. Stomach contents of *Megaleledone senoi* TAKI, n. g. et sp., ca. nat. size.
A, Thin scaly pieces of unknown origin.
B. Yellowish brown, amorphous masses of unknown origin.
C. Broken arms of Ophiuroidea (genus *Stegophiura ?*).
D. The same (subfamily Ophiomastinae).
E. The same (*Ophiura* sp.).

*Stomach* (st) rouhgly spheroidal, 55 mm wide, 42 mm long and 37 mm thick; it was tightly filled with food to form a compact mass which weighed 19 g in all. The solid contents excluding fluid (Text-fig. 7) consist of (1) chalky white calcareous pieces, ca. 5–15 mm long, which are broken arms of the Class Ophiuroidea, together with part of their discs irregularly cut; they were examined by Dr. Shirô MURAKAMI as follows: (Text-fig. 7, C): A species quite akin to *Stegophiura*, judged by the broken pieces of arms whose spines were lost; (D) A species referable to the subfamily Ophiomastinae of the family Ophiolepididae : it is nearly impossible to determine the genus, because the specimens lack the oral parts, though they are represented by the broken pieces of arms equipped with spines and broken aboral parts of discs. (E) A species referable to the genus *Ophiura*, the specimen being provided with imperfectly preserved oral parts.

(2) (B) yellowish brown amorphous, apparently gelatinous masses of unknown origin;

(3) (A) dark brown or light yellowish brown, thin transparent or semitransparent, scaly pieces of irregular sizes, of unknown origin.

It is not possible to know the nature of (2) and (3), but may be of animal matter.

*Intestine* (in): Wall very thin and soft, with about 4 loops in its entire course, the widest part about 25 mm wide, containing within numerous buff coloured small grains. They are fine, about 1 mm or less in diameter, including white grains and very short tubes. It is sure that the contents of intestine are consistently the same as those of the stomach, though in a much smaller size, before they are digested into a fluid state.

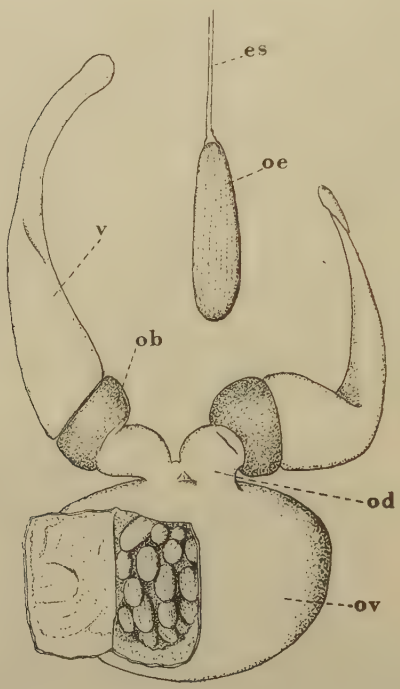
*Rectum:* About 7 mm wide, its wall is rather thick and firm in consistency, ending at a swollen part, which is ca. 14 mm wide when depressed.

*Anus* (a): No particular valve is seen on the margin of the anal opening, the margin being minutely incised; but there are three longitudinal short wrinkles on the inside of the dorsal wall.

*Female genital organ* (Text-fig. 8): Ovary large, depressed spherical in outline as imbedded in the viscera, 56 mm wide and 43 mm long; capsule rather firm, muscular, 0.5 mm thick, the interior of which is wholly invested with soft, loose delicate tissue, which is 1–2 mm thick. Female gonoduct as a whole 88 mm long, oviducal ball $11 \times 17$ mm, oviduct is rather short, ca. 8 mm long, vagina 68 mm long, widest at the proximal part (17 mm) and narrowest at the anterior 2/3 of the vagina; left vagina is shorter than the right one, while the left oviducal ball is larger than the right one.

Ovarian eggs seem to be of a matured size: each egg $5.5 \times 17$ mm (13% of the mantle-length), stalk 14 mm long, and ca. 50 eggs contained, but several smaller ones are found among them.

*Statocyst* (Text-fig. 4): examined by dissecting from the ventral side: each cyst ovoidal, 13 mm wide and 8 mm high, rounded at the inner margin and tapers outwardly; no papillae found in the interior of the cyst; there is a spherical, thin, transparent jelly-like capsule at the centre of the cyst, which contains in turn a smaller




Text-fig. 8. *Megaleledone senoi* TAKI, n. g. et sp. Ventral view of female genital organ. ×2/3.
es, egg stalk; ob, right oviducal ball; od, left oviduct; oe, ovarian egg; ov, ovary, part of its capsule cut open; v, right vagina.

spherical delicate capsule at its centre. No calcareous statolith is found. The animal has been preserved in formalin solution since its capture in 1957, but the contents of both stomach and intestine, which contained Ophiuroid skeletons, were not decalcified. It is surmised that the statolith, if it is actually present, may not have been decalcified in this specimen, as it is protected by a rather thick wall composed of a cartilage. In short, no statolith exists in this species.

*Branchial gland* (Text-fig. 5, br): the right glandular tissue, the capsule being peeled off, measures 55 mm long, 16 mm wide and 9 mm thick, weighs 4 g. The ratio to the body weight (2860 g) of the gland on both sides (8 g) is 0.00280.

## CONSIDERATIONS ON THE MORPHOLOGICAL CHARACTERS OF *MEGALELEDONE SENOI*

*Size of animal*: It is worth while to note that *Megaleledone senoi* is very great in size in comparison with the members of allied genera as shown in Table 1. Namely, members of *Eledone* is the largest and *Megaleledone* comes next, the rest is generally rather small. This is, needless to say, a mere comparison of linear measurement, and *Megaleledone* is so squat and big that in this group it may come at a high rank in the weight of animals. I cannot compare the weight as such data

Table 1. Comparison of total length, mantle length and length of longest arm in Eledoninae, Bathypolypodinae and Megaleledoninae; in each item maximum value is adopted.
* Approximate value calculated from the description or estimated from the figure on plate.

| Subfamily | Genus | Species | Total length mm | Mantle length mm dors. | Mantle length mm vent. | Length of longest arm mm | Author |
|---|---|---|---|---|---|---|---|
| Megaleledoninae | *Megaleledone* | *senoi* | 460 | 135 | 102 | 295 | TAKI 1961 |
| Eledoninae | *Eledone* | *moschata* | 170 | 45 | — | 135 | JATTA 1896 |
| | | *cirrosa* | 558[1] | 160 | — | 360 | ISGROVE 1909 |
| | *Pareledone* | *charcoti* | 96 | — | 32 | 60 | JOUBIN 1905 |
| | | // | — | — | 40 | 131 | MASSY 1916 |
| | | *turqueti* | 42 | — | 15 | 28 | // |
| | | *polymorpha* | (200)* | 56 | — | (148)* | ROBSON 1930 |
| | | *harrissoni* | 336 | 78 | — | 248 | DELL 1959 |
| | | *adelieana* | 110 | — | 25 | 76 | BERRY 1917 |
| | | *antarctica* | — | — | 100 | 180 | THIELE 1921 |
| | | *umitakae* | 120 | 45 | 36 | 73 | TAKI 1961 |
| | *Velodona* | *togata* | (430)* | 111 | — | (300)* | CHUN 1915<br>ROBSON 1932 |
| | | *togata capensis* | — | 60 | — | 78 | ROBSON 1932 |
| | *Tetracheledone* | *spinicirrus* | — | 42 | — | 81 | VOSS 1955 |
| Bathypolypodinae | *Bathypolypus* | *arcticus* | 215 | 66 | — | 78 | ROBSON 1932 |
| | | *lentus* | — | 60 | — | ?69 | // |
| | | *obesus* | — | 72 | — | 66 | // |
| | | *sponsalis* | — | 39 | — | 77 | // |
| | | *salebrosus* | 153 | — | 40 | 100 | SASAKI 1929 |
| | | *valdiviae* | — | 34 | — | 71 | ROBSON 1932 |
| | | *grimpei* | — | 40 | — | 72 | // |
| | *Graneledone* | *challengeri* | — | (93)* | — | (165)* | HOYLE 1886 |
| | | *verrucosa* | — | 100 | — | 85 | VERRILL 1881 |
| | | *verrucosa media* | — | ca. 86 | — | 76 | ROBSON 1932 |
| | | *setebos* | — | — | 170 | 220 | MASSY 1916 |
| | *Thaumeledone* | *brevis* | 55 | — | 15 | 35 | HOYLE 1886 |
| | | *gunteri* | (100)* | 36 | — | (57)* | ROBSON 1930 |
| | *Bentheledone* | *rotunda* | 165 | — | 45 | 110 | HOYLE 1886 |
| | | *albida* | 170 | 35 | — | 129 | BERRY 1917 |

1) JAECKEL (1958, p. 562) gives the maximum length 105 cm, average 38 cm.

are not available in the literature.

*Form of mantle*: In members of Eledoninae, it is more or less ovoidal or somewhat spherical, and transversely elongated form is rather rare. This species resembles *Graneledone setebos* ROBSON (ROBSON 1932, p. 313) of the subfamily Bathypolypodinae, in the broadness of mantle, though it is known by only a single, man-

gled specimen (width-index 120: this species 106).

*Radula*: In *Pareledone*, the rhachidian tooth is pentacuspid or tricuspid in *P. turqueti*, *harrissoni* and *polymorpha*, unicuspid in *adelieana* and *charcoti*, but all of them have a second lateral with a wide base and low cusp. *Graneledone setebos* Robson (cf. Massy 1916, p. 160, fig. 33), which has the most similar radula to the present species, differs from the present species by the fact that its third lateral has a rather wider base and lower cusp than this species. Robson (1932, p. 134) remarks on the radula of *Graneledone* that "the simplicity and uniformity of the teeth being in my opinion definitely archaic."

In the subfamily Bathypolypodinae, in *Bathypolypus articus* and *B. sponsalis* the rhachidian is unicuspid, but the first and second laterals are very wide, in *Graneledone challengeri* the rhachidian is tricuspid and the second lateral has a wider base in comparison with *Megaleledone senoi*.

The radula of *Megaleledone senoi* is characterizd as follows:

(1) rhachidian unicuspid;
(2) first lateral is equipped with a well-developed cusp;
(3) second lateral has a comparatively narrow base and long cusp;
(4) third lateral has a narrow base and rather straight cusp;
(5) marginal plate missing.

In the present species, the mandibles, radula, stomach, liver and intestine are well-developed; especially the presence of as much food matter as the stomach can contain and also the fact that the whole body is muscular with arms having rather large suckers show that the animal is quite active and voracious in habit.

The wideness of mantle opening is related to the littoral habit of the animal (Robson 1926).

*Food*: Though the octopod is predatory and carnivorous in its food habit, the range of food is rather wide (Foraminifera, Polychaeta, Crustacea, Mollusca, and fish, exceptionally sea-weed: Massy 1916; Robson 1929, p. 20; 1932, p. 22). However, so far as I am aware, there has been no record of Ophiuroid animals as food of Eledonid octopods. The present case may be an unusual food for the Octopoda in general, and it is surmised that the scarcity of fleshful animal species of a habit of sluggish movement in the Antarctica may have compelled this species to search animals having scanty flesh.

*Alimentary canal*: The absence of crop and the smallness of the posterior salivary gland, spiral caecum and ink-sac seem to suggest that the animal in question has a tendency to be of deep-sea form (cf. Robson 1926). However, no substantial atrophy is seen in the respiratory as well as circulatory organs. The branchial gland which is an organ of internal secretion (Taki 1943) is of a normal or rather large size, and its ratio to body weight is a little greater than that of *Octopus vulgare*. I formerly showed that in three species of *Octopus* its ratio is greater in the larger species approximately proportionally to its body weight, and also it is greater in female than in male. Though this is a single case and the range of variation is not known, the fact that a large species and also a female is equipped with a heavy

branchial gland agrees well with the result observed in the genus *Octopus*. A conjecture might be allowed from the comparison of these figures that the branchial gland has an important bearing on the maturation of the ovary.

Ratio of branchial gland weight to body weight. Female.

| *Megaleledone senoi* | *Octopus vulgare* | *O. minor variabilis* | *O. ocellatus* |
|---|---|---|---|
| 0.00280 | 0.00247 | 0.00191 | 0.00156 |

*Female genital organ*: The ovary with its matured eggs, oviducal ball and vagina of this species are of a great size not comparable to any of those of hitherto known species of subfamilies Eledoninae and Bathypolypodinae. The greatness of the ovarian egg leads necessarily to the reduction in the number of eggs, and it is

Table 2. Comparison of characters of four subfamilies of Octopodidae.

| Characters | | Octopodinae | Eledoninae | Bathypolypodinae | Megaleledoninae |
|---|---|---|---|---|---|
| Form of mantle | | oblong or oval | oval or spherical | oval or transversely oval | transversely oval |
| Mantle aperture | | wide | wide | narrow | wide |
| Ink-sac | | present: well-developed | present: well-developed | absent | present: abortive |
| Arm-length: index | | long or moderately long 63–90% | moderately long 67–81% | short or rather short 63–85% | short 64% |
| Sucker | arrangement | biserial or triserial | uniserial | biserial or uniserial | uniserial |
| | size | large | large | small | large |
| | index | 5–14% | 8–11% | 4–10% | 11% |
| Web-depth: index | | shallow 4–42% | shallow 16–40% | deep 25–68% | deep 44% |
| Crop | | present | present | reduced or absent | absent |
| Eggs and vaginae | | small | small | large | large |
| Radula | rhachidian | penta-, tri-, or unicuspid | penta-, tri-, or unicuspid | unicuspid | unicuspid |
| | 1st lateral | cusp low, base wide | cusp low, base wide | cusp low, base wide, or cusp high, base narrow | cusp high, base narrow |
| | 2nd lateral | cusp high, base wide | cusp high, base wide | cusp high, base wide or narrow | cusp high, base narrow |
| | 3rd lateral | cusp long, curved | cusp long, curved | cusp long, curved | cusp long, straight |
| | marginal | present | present | absent | absent |
| Depth of habitat | | mostly littoral | mostly littoral | abyssal | archibenthal |

legitimately expected that an exceedingly large young will hatch out from such an egg.

The large size of the oviducal ball and oviduct may perhaps have a close relation to the production of large eggs.

The oviducal system is of asymmetrical construction in the Cirrata, "the left hand members alone are found (ROBSON 1932, p. 15), while it is symmetrical in Incirrata which includes the majority of Octopod species. In the present species the right oviducal system is longer than the left one. It may, therefore, be able to surmise that the asymmetrical structure found in this species is assigned to the transitory state toward the bilateral differentiation of the oviducal system, though I could find no particular differentiated structure in other organs in this species.

*Subfamily relation*: Though this animal was caught at a depth of archibenthal fauna, it morphological characters are nearly littoral and only a little modified for the adaptation to the life of deep seas. Characters of *Megaleledone* such as the mantle-aperture, sucker arrangement, gill size and number of filaments, have in common with the subfamily Eledoninae, but differ from it in the ink-sac, arm-length, crop, eggs and vaginae, web-depth, etc. While they are similar to subfamily Bathypolypodinae in the outline of mantle, arm-length, crop, eggs and vaginae and web-depth, but fairly different in the mantle aperture, gill size and number of filaments.

The radular characters of this species somewhat resemble those of *Graneledone setebos*, which fact may show a racial affinity to that species, but it is difficult to place this species in Bathypolypodinae owing to various points of difference as given in Table 2.

In conclusion, it seems appropriate to establish a new subfamily to receive the present species as follows:

Subfamily Megaleledoninae, new.

Characters of the genus *Megaleledone*, n. gen., as given above.

## Subfamily Eledoninae

### *Pareledone umitakae* TAKI, new species

(Pl. 3; Text-figs. 9–16)

Two specimens, both males.

Locality, Date and Gear the same as *Megaleledone senoi*.

*General appearance*: Animal firm in consistency, but integument thin, soft and choroidal, mantle as broad as long; eye-ball extraordinarily voluminous, as seen from the dorsal side it is 15 mm long and 15 mm wide, leaving a narrow (ca. 6 mm) interspace between both eyes; a small ocular cirrus, 2 mm long and 3 mm high, is found at the posterior corner of the right eye-ball, the integument of the left eye being scraped off; arms short and slender as compared with the head and body taken together. Surface of integument apparently smooth, though weak, thin reticular wrinkles are discernible all over the surface under a lens.

*Colour* greyish reddish brown; a number of reddish brown patches are scattered

over the surface of which a large diamond-shaped (3.5 × 5 mm) patch is situated at the centre of the dorsal mantle, and a smaller one (2.5 × 3.5 mm) between both eyes, a smallest one (4.5 × 1.5 mm) at the middle of the dorsal side of eye-ball, and an irregular-sized one (3 × 3 mm) between the anterior margin of both eyes; numerous minute patches (ca. 0.5 mm in diameter) are sparsely scattered all over the surface of body and arms. Ventral surface of head, mantle, arms and webs is light greyish purplish red.

*Mantle*-aperture very wide; no peripheral keel.

*Funnel-organ* (Text-fig. 13) W-shaped; the central part rather wide (2.5 mm), but anterior parts of outer and inner limbs taper up to the extremity.

*Gill*-leaflet 8 in both the inner and outer demibranchs on each side.

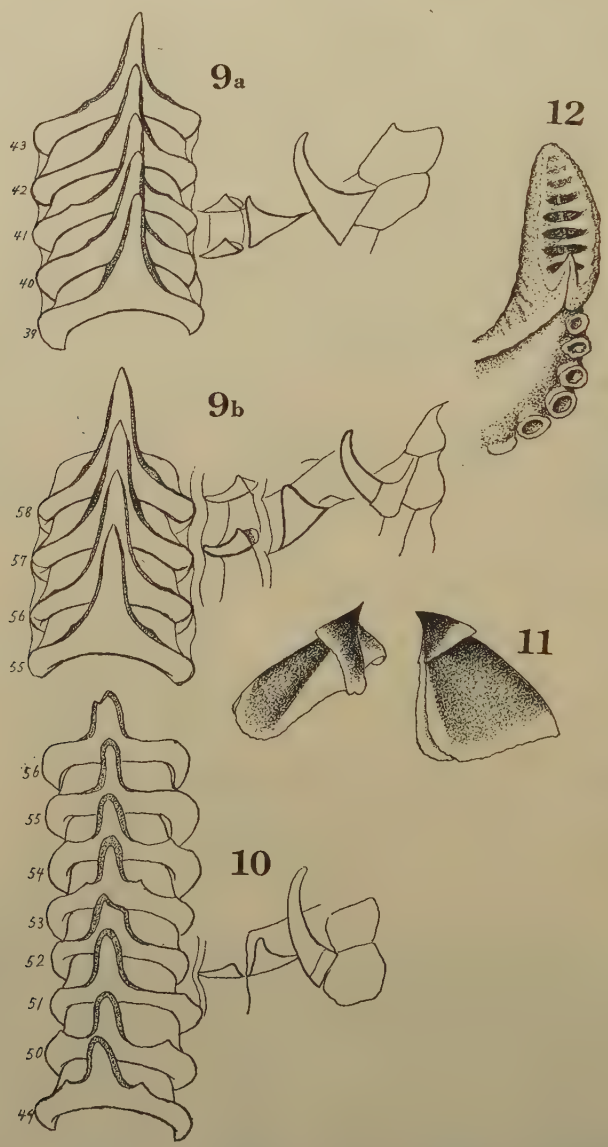
*Upper mandible* (Text-fig. 11b): Rostrum blackish brown, 1.7 mm long, rather sharply pointed, with a thin margin posteriorly, palatine lamina dark brown, with a thin margin ca. 1 mm in width. Lower mandible (Text-fig. 11a): Rostral wing narrow, ca. 2.2 × 3.3 mm, protruded straight, not curved, jugular lamina quadrangular, a notch at the posterior centre, with a rather wide thin margin on both sides.

*Radula* (Text-fig. 9): Nearly symmetrical; rhachidian unicuspid, base very wide,

| Specimen | | A | | B | |
|---|---|---|---|---|---|
| Total length | | 120 mm | | 37 mm | |
| Body weight | | 61 g | | 2.8 g | |
| Mantle-length | | 45(dors.), 36(vent.)mm | | 15(dors.), 13(vent.)mm | |
| Mantle-width | | 35 mm | | 15 mm | |
| Head-width | | 36 mm | | 14 mm | |
| | | L | R | L | R |
| Arm length | 1 | 65 | 63 | 20 | 21 |
| | 2 | 67 | 73 | 22 | 22 |
| | 3 | 70 | 63(h) | 22 | 22 |
| | 4 | 73 | 73 | 21 | 20 |
| Arm formula | | 4. 3. 2. 1. | | — | |
| Web depth | A | 18 | | 7 | |
| | B | 19 | 21 | 7.5 | 7 |
| | C | 20 | 21 | 8 | 8 |
| | D | 21 | 23 | 7 | 7 |
| | E | 17 | | (broken) | |
| Diameter of largest sucker | | 4 mm | | 0.9 mm | |
| Arm-length index | | 61% | | 59% | |
| Mantle-width index | | 78% | | 100% | |
| Head-width index | | 80% | | 93% | |
| Web-depth index | | 31% | | 36% | |
| Mantle-arm index | | 62% | | 68% | |
| Sucker-index | | 5.5% | | 4.1% | |
| Ligula-index | | 12.7% | | — | |

with a rather long and acutely pointed cusp; a nearly inconspicuous swelling at the position of ectocone, which fades away gradually in a manner of $A_3$ seriation; first lateral very small, with a low triangular cusp at the outer side; second lateral rather low, with a flat, triangular cusp; third lateral small as compared with the rhachidian, base wide, moderately curved distally; marginal plate very thin, roughly hexagonal.

*Arm*: Length is subequal, especially in specimen B, its order 4.3.2.1 on the left side, and the index 61%. Suckers uniserial.



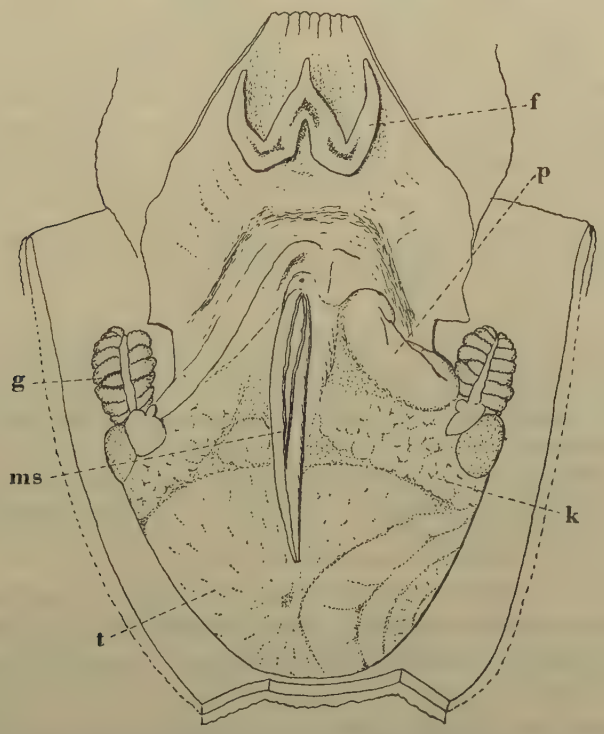

Text-figs. 9–12. *Pareledone umitakae* TAKI, n. sp. 9a, anterior; 9b, middle part of radula, ×65; 10; radula of young specimen, ×260; dotted area shows a thin blade on both sides of the cusp; 11, mandibles, ×3.3; 12, hectocotylus, ×2.6.

*Web*: Narrow, its index 31%, its order D.C.B.A.E.

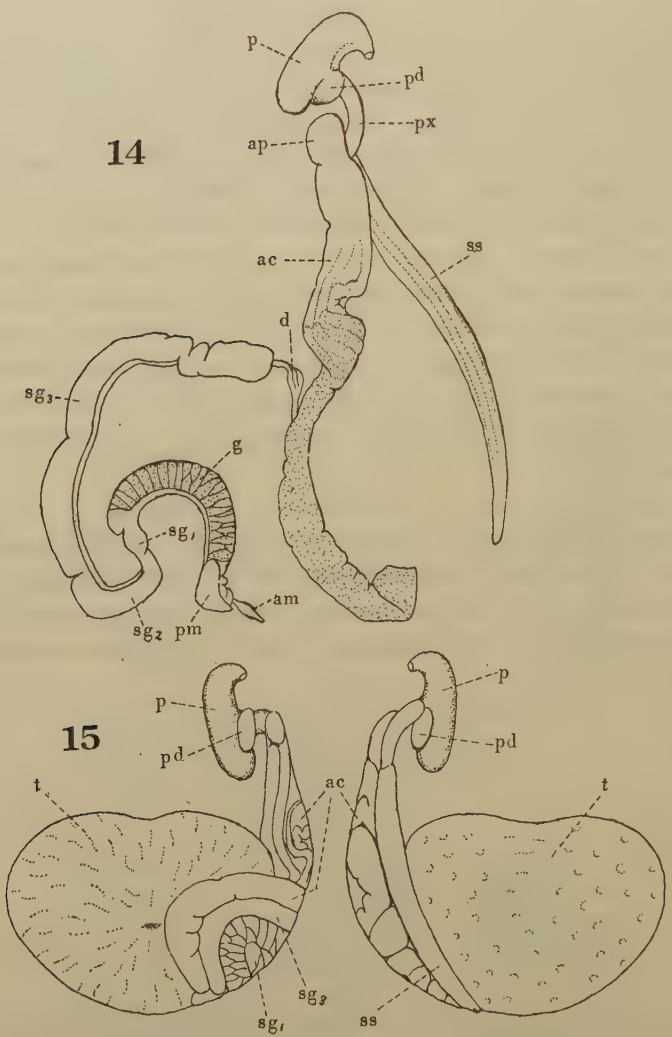
*Hectocotylized arm* (Text-fig. 12): Shorter than the arm on the opposite side; suckers number 28 in all, ligula 8 mm long and 4 mm wide, 8 very deep transverse grooves separating 7 bars; calamus 2 mm long. Ligula index 12.7%.

*Male genital system* (Text-figs. 14–15): Testis. When the male gonoduct system is liberated, the testis is roughly trigonal and depressed, the hilus portion being at the centre of the ventral side of it. Gonoduct. Proximal portion of vas deferens is slightly swollen (ampulla); in the succeeding part both the proximal massive region (pm) and the glandular part (g) seem to correspond to the vas deferens plus proximal spermatophoric gland in *Octopus* (cf. TAKI 1944). Next comes a rather smooth-walled spermatophoric gland (sg) of nearly equal caliber, which turns thrice, the distal part being widened in diameter. This part may be divided into three regions, namely the first, second and third ($sg_{1\sim3}$) according to the turning points and the internal structure.

The spermatophoric duct (d) is thin-walled and wrinkled. The accessory spermatophoric gland (ac) is glandular except the distal part, which is rather thin-walled and wrinkled longitudinally; the proximal part is rather flattened and abruptly crooked on the left side as in *Octopus*. Next, the "shunting duct" (ap) is nearly spherical, not pointed anteriorly. This is generally termed "spermatophoric duct," but it




Text-fig. 13. *Pareledone umitakae* TAKI, n. sp. Ventral view of viscera in situ, mantle and funnel cut open, ×2; f, funnel-organ; g, gill; k, kidney; ms, mantle septum; t, testis.




Text-figs. 14–15. *Pareledone umitakae* TAKI, n. sp. 14. Male gonoduct system, ×2. 15. Male genital organ, ventral and dorsal views, in situ. ×1.7.
ac, accessory spermatophoric gland; am, ampulla; ap, shunting duct; d, spermatophoric duct; g, glandular part of vas deferens; p, penis; pd, penis diverticle; pm, proximal part of vas deferens; px, proximal part of penis; $sg_1$, $sg_2$, $sg_3$, first, second and third regions of spermatophoric gland; ss, spermatophoric sac; t, testis.

is better to call it a shunting duct to avoid confusion with the same term which denotes the duct between spermatophoric gland and accessory spermatophoric gland. The spermatophoric sac is slender and generally tapers behind, no spermatophore is found within.

The proximal part of penis (px) is thick-walled, and penis diverticle (pd) rather small; penis (p) muscular and remarkably thick-walled, the distal part gently curved, with a small aperture at its end.

Table 3. Comparison between *Pareledone polymorpha* and *P. umitakae*.

| Characters | *polymorpha* | | *umitakae* |
|---|---|---|---|
| | forma *oblonga* | forma *affinis* | |
| Peripheral ridge of mantle | present | | absent |
| Radula {second lateral | large, wide | | small, narrow |
| Radula {third lateral | long, wide | | short, narrow |
| Web-depth | 45–35% | 30–27% | 31% |
| | A=E | — | A>E |
| Arm | 72–65% | — | 61% |
| Sucker | 7–8% | 11.6–7% | 5.5% |
| Hectocotylus | 15–13.9% | 17–9% | 12.7% |
| Mantle | narrow and oblong | wide | rather narrow |
| | 75–64% | 104–70% | 78% |
| Head-width | 69–61% | 90–53% | 80% |
| Type of mantle-aperture | B | | A |

*Specimen B.* Radula (Text-fig. 10): Rhachidian $A_4$ seriation, first lateral very low, otherwise similar to the adult specimen. But it is noticeable that the tip of the main cusp of rhachidian is shallowly bifid (Nos. 52 and 56), and each cusp is followed by a low mesocone of the next tooth. In the posterior part of the radula the rhachidian cusp is longer and more pointed.

ROBSON (1929, p. 14) did not "attach very great weight on the seriation in the diagnosis of species..." and says that "the rhachidian tooth of young forms may have different seriation that found in old ones." Namely, no remarkable difference is found between radulae of specimens A and B.

Mandibles: So thin and weak in consistency that at dissection they were partly broken; lower mandible 2.3 mm long and upper one 2.5 mm long. Form as that of the adult.

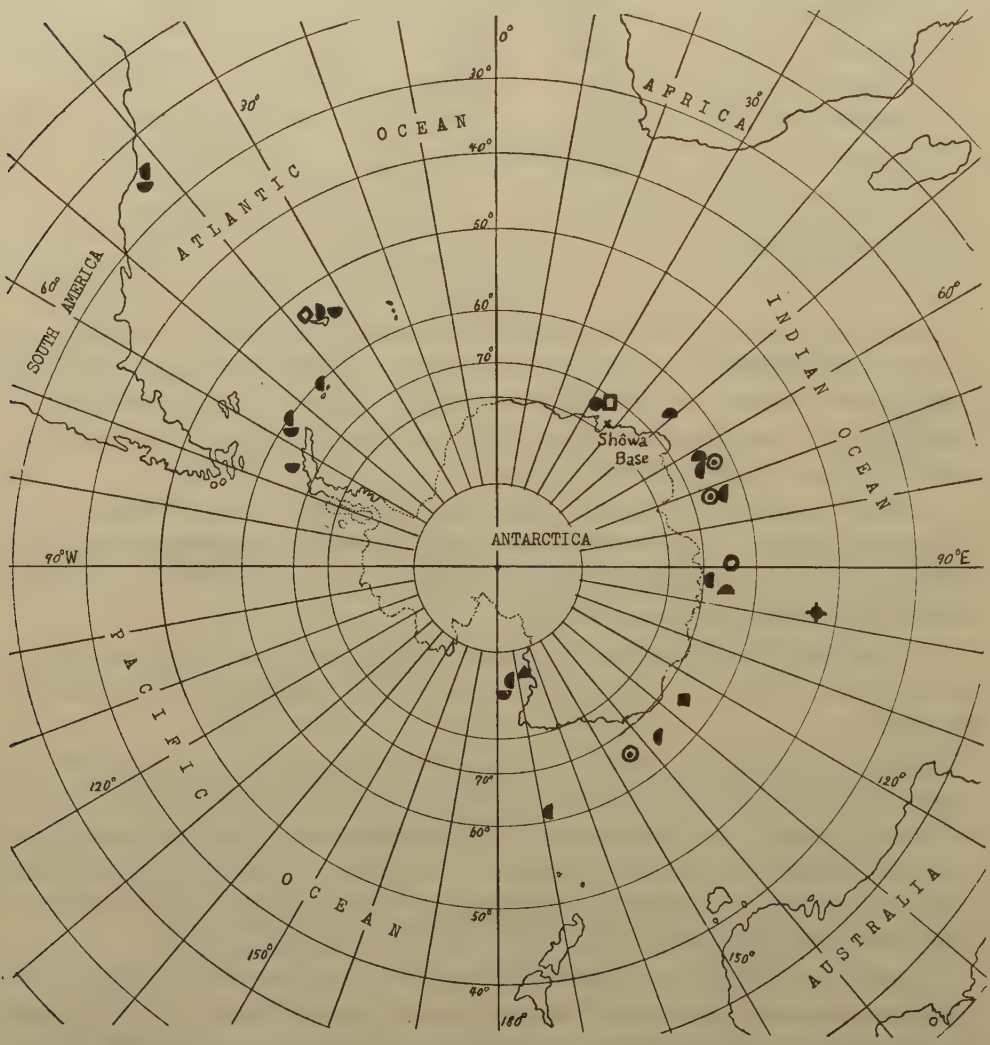
In this specimen the fact that (1) the colour of body is light, (2) body and integument is soft, (3) the arm is disproportionally short, (4) mandibles are soft and not thickened, (5) radula, (6) hectocotylus and (7) genital organs are in an undeveloped state, shows that it is a juvenile specimen. Also by the fact that it was obtained at the same haul as the specimen A, and radula, mandible, six kinds of indices of body proportions agree well with it, it is identified with this species.

*Remarks*: This species is placed in the genus *Pareledone* by the presence of unmistakable ligula and calamus. By the W-shaped funnel-organ this species differs from *P. charcoti* (= ? *aurorae*), *turqueti* and *harrissoni* which have VV-shaped one. *P. nigra* has very long arms (78%) and large (15%) suckers, and coloured nearly black; *P. adelieana* has a larger second lateral than the present species. In *P. antarctica* the suckers "have a zigzag arrangement, and are arranged far more like those of an *Octopus*" (ROBSON 1932, p. 280); also the arm is slightly longer (66–62%), hectocotylus is shorter (4.2%) than the present species; the order of web-

depth is E>A in *antarctica* while A>E in the present species.

Though many points of resemblance are found between *P. polymorpha* and this species, the following may be sufficient to distinguish the two, namely,

(1) surface of body smooth, not tubercular;
(2) peripheral ridge of mantle is absent;
(3) mantle aperture is very wide;
(4) arm is shorter;




□ Megaleledone senoi
⊙ Pareledone adelieana
○ Pareledone antarctica
◖ Pareledone charcoti
◓ Pareledone harrissoni
◗ Pareledone polymorpha
◡ Pareledone turqueti
● Pareledone umitakae
▲ Graneledone setebos
◇ Thaumeledone gunteri
■ Bentheledone albida
✦ Bentheledone rotunda

Text-fig. 16. Map of the Antarctic and Subantarctic Regions in which localities of species of Eledoninae, Bathypolypodinae and Megaleledoninae occurring therein are shown.

(5) sucker is smaller;

(6) second and third laterals of the radula smaller and narrower, as compared with that species.

This species is named in honour of the research vessel "Umitaka-maru" which brought back this specimen.

## ZOOGEOGRAPHICAL CONSIDERATIONS

Cephalopod species hitherto known from Antarctic and Subantarctic Seas are recently listed by POWELL (1960), and localities of those belonging to subfamilies Eledoninae, Bathypolypodinae and Megaleledoninae are shown in Text-fig. 16.

The seas where both new species herein described were obtained have long remained unexplored by any expedition owing to their extremely unfavourable meteorological conditions for approaching all the year round. DELL (1959) subdivided the shores of the Antarctic Continent into 5 areas according to the longitude. According to him, two new species described here are included in the '5th area.'

I have already shown that *Graneledone setebos* is somewhat related to *Megeleledone senoi* by the radular character which hold an archaic feature, but the locality of the former is situated at nearly the opposite side of the Antarctica to that of the latter. Namely, at present *Megaleledone* stands solitary zoogeographically.

Seven species are known in the genus *Pareledone* (ROBSON 1932), of which six species are confined to the Antarctic and Subantarctic Seas, and one species *P. nigra* occurs in South-west Africa. I have shown that *P. umitakae* is somewhat related to *P. polymorpha*, but the localities of both species are, so far known, very distantly separated, especially *P. umitakae* was obtained at a higher latitude than *P. polymorpha*. However, both *P. charcoti* and *P. turqueti* have a very wide range of distribution, the former covering 1–5 areas and the latter 1–3. In general, members of this genus are widely distributed in the South Atlantic, Subantarctic and Antarctic Seas and occurrence of species of an endemic nature at a particular habitat is rather rare, though our knowledge of the distribution is as yet quite unsatisfactory.

Members of *Pareledone* inhabit the sea of a wide vertical range: both *P. turqueti* and *P. charcoti* ranges from 24 m to ca. 1300 m; *P. nigra* in a shallow sea, *P. polymorpha* 15–270 m, *adelieana* and *harrissoni* ca. 600 m (*antarctica*, depth unknown). Thus, *P. umitakae* belong to a rather deep-sea inhabitant in this genus, which fact agrees with the softness of integument of this animal.

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## EXPLANATION OF PLATES 1—3

Pl. 1. *Megaleledone senoi* TAKI, n.g. et sp. Dorsal view, × ca. $\frac{1}{3}$.

Pl. 2. The same, ventral view.

Pl. 3. *Pareledone umitakae* TAKI, n. sp..

Figs. 1, 2. Dorsal and ventral views of adult specimen, ca. nat. size.

Figs. 3, 4. Dorsal and ventral views of young specimen, × ca. 1.5.

TAKI: Eledonid Octopods from Antarctic Sea

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# Studies on the Winter Eggs of the Water Flea, *Moina macrocopa* Straus.

Yutaka Murakami

*Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Plates 1–2; Tables 1–3, Text-figs. 1–16)

## (I) INTRODUCTION

The family Daphnidae contains many of the most important water fleas found in the fish culture ponds in Japan. Among others *Moina macrocopa* occupies the first place, followed by *Daphnia pulex*. In Japan the artificial propagation of *Moina macrocopa* has a great importance, because it is the most desirable and nutritious natural food for newly hatched fry in the carp-culture farm. It has been also used as a good material for ecological study of the Cladocera in general.

Many works have been reported on the biology of the Cladocera, but most of them are related to their parthenogenetic or asexual reproduction and little was added to the knowledge on their sexual reproduction and sexual eggs produced in the ponds. Therefore it is much desirable that a survey be made on the degree of relationship existing between the stock of winter eggs (sexual eggs) and the population growth.

Sexual reproduction of *Moina macrocopa* takes place at mid-May in the carp-culture ponds of the Kanto district and its winter egg production is also seen during last ten days of that month. Moina disappears in the ponds from June to April of the following year and it spends as long as about eleven months in the form of the winter eggs at the bottom of the ponds. This phenomenon is called "Sleeping" of the winter eggs.

Hence, the present studies have been carried out on the winter eggs of *Moina macrocopa* to make clear the mechanism of the sleeping.

Before going further, the author is happy to express his thanks to Professor emeritus, Dr. I. Amemiya and Prof. Dr. Y. Oshima for their guidance during the progress of this work. Further he wishes to express his thanks to Prof. Dr. Y. Matsue and Ass. Prof. Dr. S. Egusa for their valuable suggestions on many matters. And lastly acknowledgement is also made to Mr. T. Onbe for technical assistance in these experiments.

## (II) MATERIAL AND METHODS

Present study was carried out by using the ponds of the Gunma Prefectural Fisheries Experimental Station in the Kanto district during these ten years from

1949 to 1959. But material was collected not only from the above mentioned ponds but also from those distributed in many prefectures, Tokyo, Shizuoka, Okayama, Hiroshima and Yamaguchi.

Experiments were made by using many winter eggs obtained from both the ponds and the small aquaria in the laboratory, because the ponds were not uniform in their character and not quite suitable for exact study.

For the purpose of analyzing the population of Moina in the ponds, samples were collected at the eight stations situated to the marginal region of the pond by a horizontal towing of a small plankton net with its mouth 10 cm. in diameter for the distance of 2 meters once a day for ten days.

In order to examine the distribution of the winter eggs in the pond bottom silt they were collected at 23 stations by using a cylinder mud sampler (10 cm. in diameter) and then counted.

The points which were observed with particular attention were as follows:

1) Growth form of population 2) Type of winter egg formation 3) Distribution of eggs in the pond 4) Non existence of pseudo-sexual egg 5) Mechanism of sleeping 6) A parasitic fungus, *Lagenidium* sp. 7) Technique of long time preservation of eggs.

## (III) OBSERVATIONS

### 1. *Moina macrocopa* in the fish culture pond.

The accordance between the peak of the population growth of Moina and the hatching time of fish is one of the most important problems in the fish culture, but both of them are not always stable as we know that they are under the influence of climate and other environmental factors. In the Kanto district spawning of carp takes place usually early in May when the water temperature is about 20°C and the hatching is expected a week later. Accordingly the pond must be prepared for Moina culture at mid or last ten days of April. For this purpose some preparation are usually taken practically, that is, exposure, lime-sprinkling and fertilization of the pond bottom.

It is well known that the Cladocera have two different generations and there is the alternation of generations, such as, acyclic, monocyclic, dicyclic and polycyclic. But our informations on the conditions and environmental factors controlling the cycle of generations of the Cladocera are not sufficient. Is Moina monocyclic or polycyclic in the ponds? The answer is not so easy, but as is above mentioned, it may safely be said that Moina in the pond of Gunma Prefecture is monocyclic, because as is shown in Figure 1 (A) it appears in the pond mostly only in May and it keeps on to early June only occasionally.

### 2. Form of population growth of Moina in the pond.

It is well known that quantitative and qualitative investigations are necessary to know the course of population growth. For this purpose the following factors relating to population-increase or -decrease were taken up and their fluctuations were

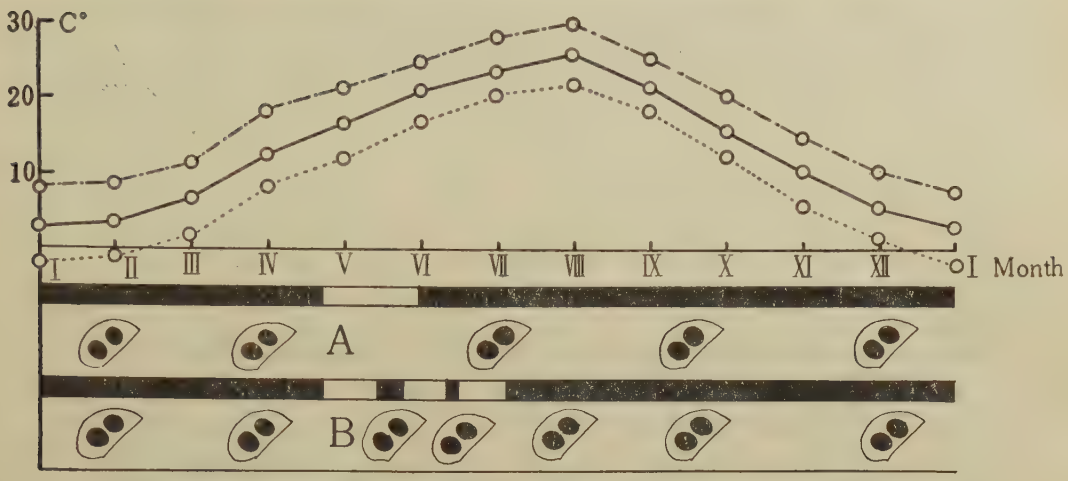


Fig. 1. Moina macrocopa STRAUS in the carp-culture ponds of the KANTO DISTRICT.

A : Monocyclic.
B : Polycyclic.

White parts mean the propagation of Moina.
Black parts mean the disappearance of Moina from the ponds, but many Winter Eggs are found at the bottom of the ponds.

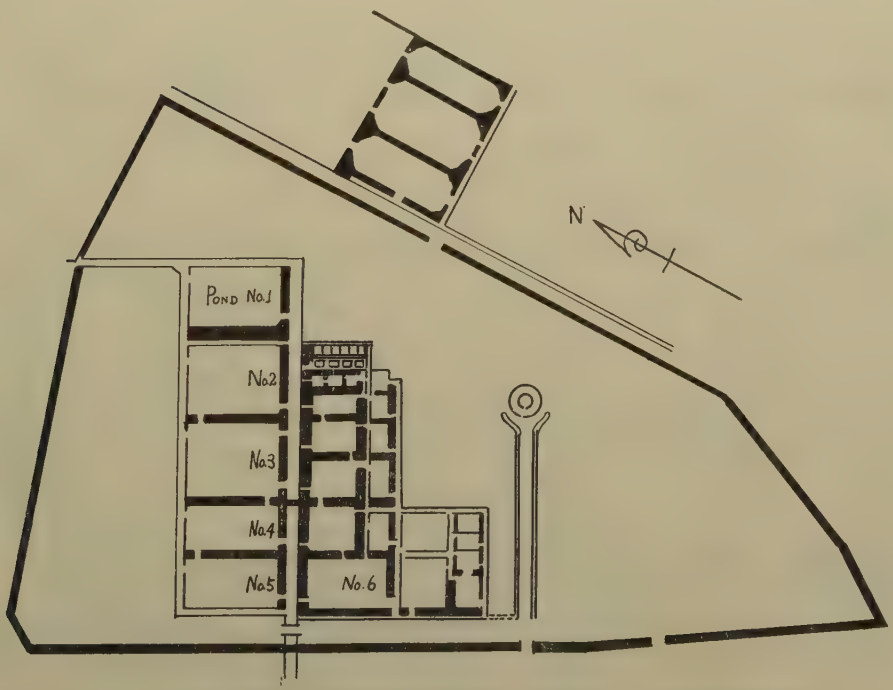


Fig. 2. A plane figure of Gunma Prefectural Fisheries Experimental Station

Pond No. 1 890m² No. 2 990m² No. 3 990m² No. 4 630m²
No. 5 730m² No. 6 580m²

observed, i.e. body size composition, sex ratio, fecundity of parthenogenetic females and the ratio of sexual females (ephippial female) to parthenogenetic ones.

This investigation was carried out during the period from May 16th to 27th of

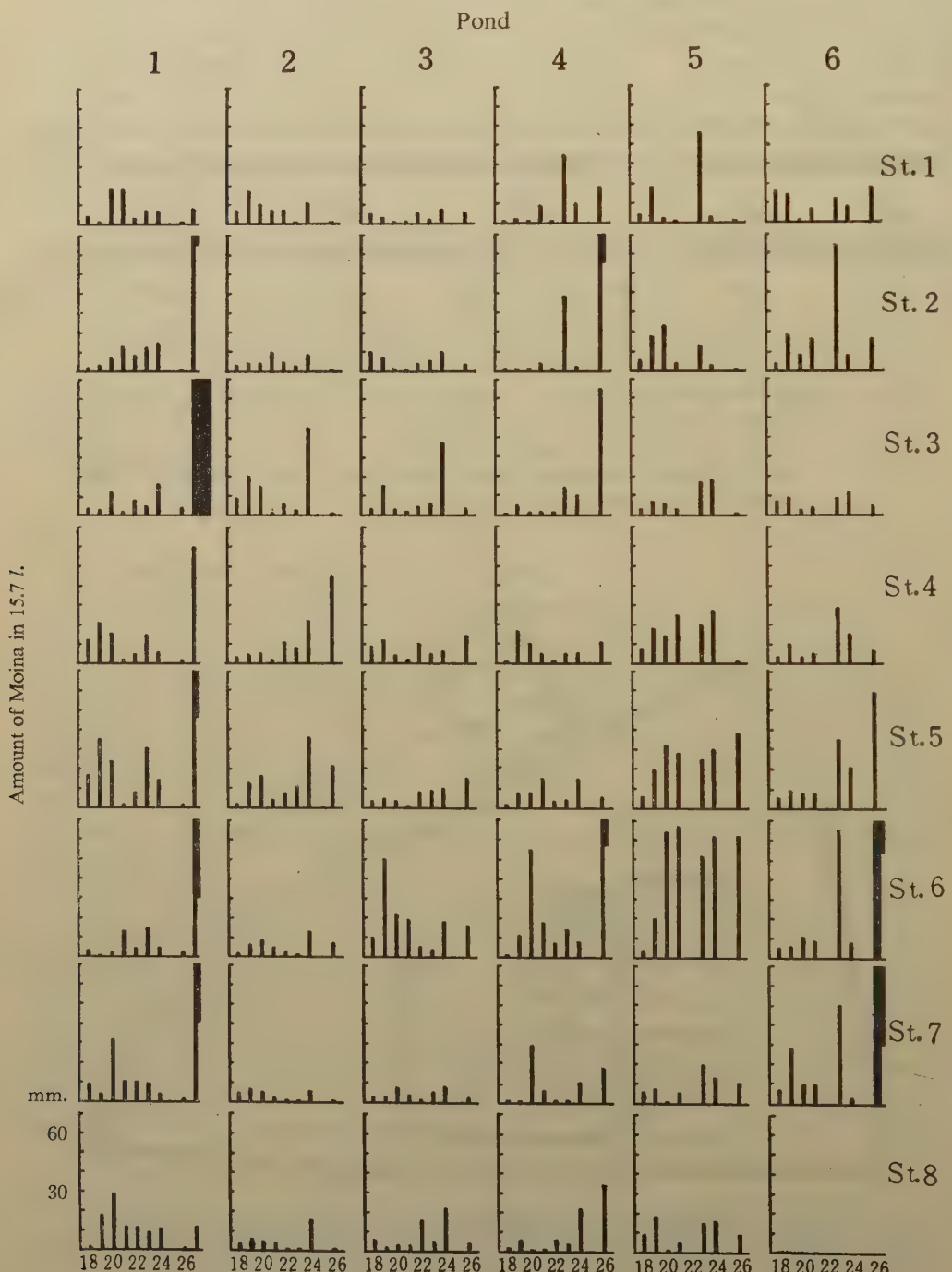


Fig. 3. Quantitative analysis of the population of Moina in the ponds.

Table 1. Average value of the amount of Moina distributed at eight stations of the six ponds of Gunma Prefecture.

| Pond No. / Station | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 |
|---|---|---|---|---|---|---|
| | mm. | | | | | |
| 1 | 7.4 | 7.6 | 3.8 | 9.5 | 10.7 | 10.9 |
| 2 | 7.9 | 5.0 | 5.9 | 16.6 | 9.7 | 19.0 |
| 3 | 6.8 | 13.8 | 9.1 | 12.3 | 7.9 | 7.0 |
| 4 | 9.6 | 12.8 | 7.6 | 6.9 | 16.0 | 10.1 |
| 5 | 16.6 | 13.9 | 6.9 | 7.6 | 25.6 | 20.9 |
| 6 | 5.6 | 5.8 | 16.9 | 24.8 | 47.0 | 37.1 |
| 7 | 10.0 | 3.9 | 4.3 | 8.4 | 8.9 | 41.0 |
| 8 | 11.9 | 4.6 | 7.6 | 10.7 | 10.4 | — |

These values mean the amount of Moina in 15.7 *l*. The value of 1 mm means 78.5 $mm^3$ in volume (=0.08 gr.)

1956, when a remarkable fluctuation of population growth was observed. Data were shown in Table 1 and Fig. 3. Samples were obtained by the above mentioned method and they were filtrated through a small wire gauze of quantitative filter (10 mm. in diameter, 100 mm, in lengrh). In Fig. 3 population size is represented in millimeter, in this case 1 mm. corresponds to 78.5 cubic millimeter in volume, and its value means the amount of Moina in 15.7 litre. Fig. 3 shows the pattern of the distribution of Moina at the eight stations in the six ponds. Granted that these figures are representing the population size, it will be able to discuss the differences of the character of distribution of Moina in the ponds.

Following results were obtained:

(1) Alarming changes in abundance have been observed in some ponds.

For instance, in the pond No. 1 all stations except St. 1 and St. 8 showed a remarkable increase on 27th, while on 26th we could not find any Moina at all stations, but the next day they appeared again in great abundance. Notwithstanding the sudden disappearance of the Cladocera have been observed by many investigators, the fluctuations between the 26th and 27th in the pond, No. 1 must be thought as a remarkable change in abundance. 350 mm. of St. 3 was the maximum value gained during this investigation and it may be safely said that it is nearly the maximum density of natural population of Moina. These phenomena were observed partially in other ponds, for example, Pond No. 2, No. 4 and No. 6 showed the same fluctuations on 24th and on 26th respectively.

(2) Evidence of the independence of the stations.

Regarding this problem the pattern of the Pond No. 5 indicated some interesting figures at St. 5 and St. 6, where the fluctuations were rather small and the population had been maintained at high level during ten days. This was proved by the data of 26th when other stations showed low value but the St. 5 and St. 6 showed the same high value of the day before.

The distribution of Moina in the ponds that were estimated to have the same condition of location, as a whole, did not indicate the same pattern. Accordingly

it is rather difficult to find a universally applicable model of the distribution of Moina in the ponds.

(3) Standard population size was estimated.

As above mentioned the distribution of Moina shows different pattern in the six ponds, but standard population size was estimated from Table 1 where average value of the samples collected at eight stations of six ponds during ten days were recorded.

It shows that the value of 20 mm. obtained by the quantitative wire-gauze sampler indicates a dependable population size demanded by fish farmer in this country.

(4) Size composition, ratio of ephippial female to parthenogenetic female and the fecundity of parthenogenetic females.

Fluctuation of size composition of Moina in the ponds must be clarified, because number of individuals do not indicate the true population size of Moina. Number of individuals is the generally adopted expression of population density, nevertheless it does not indicate the true amount of Moina in the pond, because in the cladoceran crustacea the population is constituted of individuals of various size, larva and mother. For instance, in this expression as they are similarly counted there is no difference between the mothers which have many larvae in the brood chamber and the larvae just hatched. And the population density expressed in number of individuals indicates occasionally decuple value, in spite of the same volume.

Sample was obtained from eight stations of each pond once a day, and it was divided into four size-classes, A, B, C and D by using filters of different meshes.

Four size-classes of body length are as follows:
A: $>$1.2 mm., B: 1.0–1.2 mm., C: 0.8–1.0 mm., D: $<$0.8 mm.
After their volume was measured through above mentioned quantitative filter, their percentages in volume were calculated.

In order to make clear the ratio of the number of ephippial females (sexual female) to parthenogenetic ones they were examined microscopically. As the results it was known that A, B, C and D were respectively constituted of parthenogenetic and ephippial mother females, ephippial females and large size males, inmature small males and females, and the smallest male and female larvae.

As the population-increase or -decrease factors, the fecundity of the parthenogenetic females must be examined. Then the number of larvae of summer eggs in the brood chamber of mother female were counted under the binocular microscope. Obtained data were shown in Fig. 4.

Several noteworthy results were obtained as follows:

(i) Three types of fluctuations of size-composition were observed in the population of Moina in the pond.

In the first type the difference in volume between the groupe of A plus B and the group of C plus D is large and it shows the steady growth. Consequently the population is constituted only of ephippial mother group. Pond No. 1, No. 2, No. 5

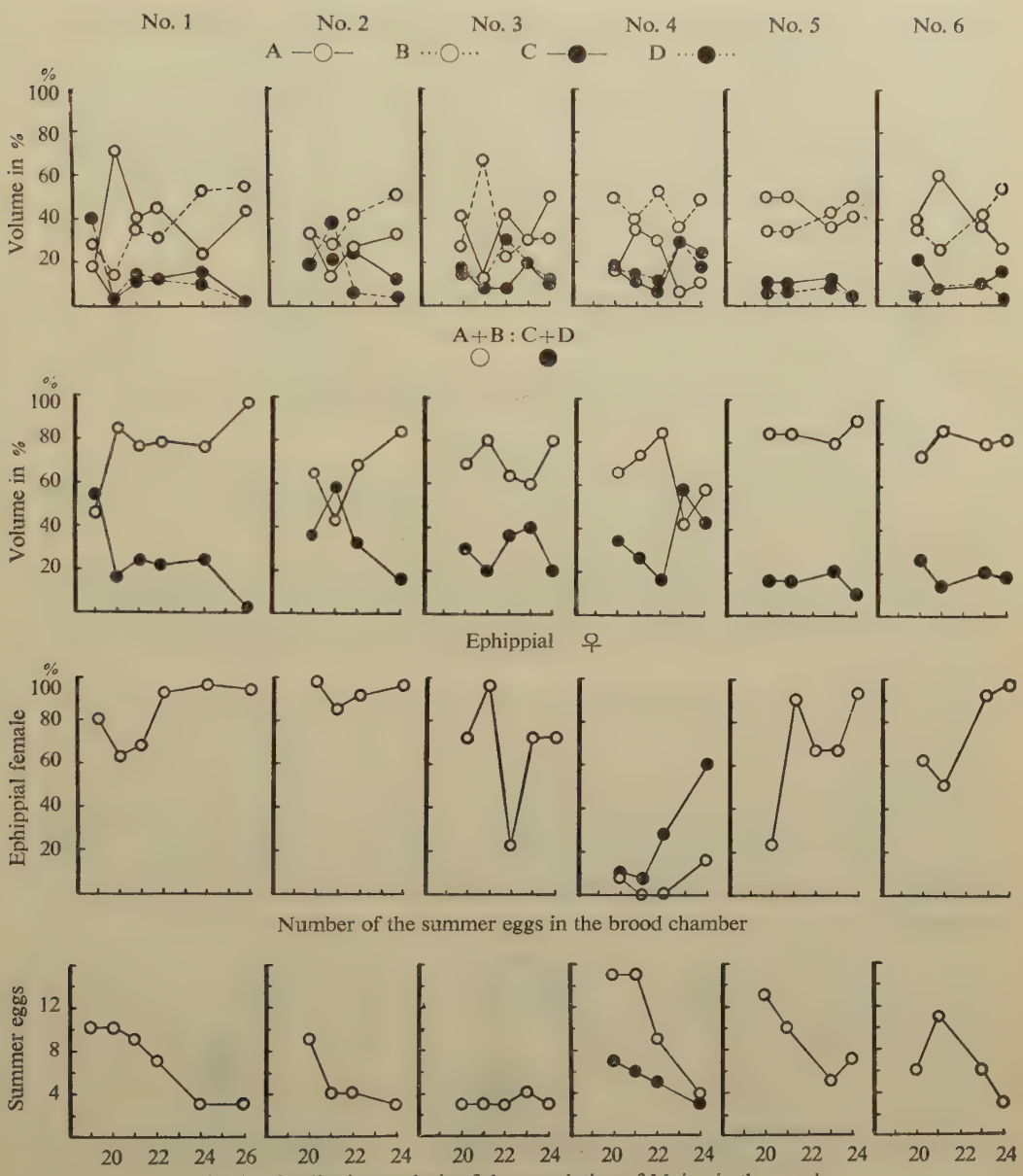


Fig. 4. Qualitative analysis of the population of Moina in the ponds.

and No. 6 belong to this type.

In the second type the difference between two groups is not so large as in the first type, and the fluctuations are wavy. In this type if it goes typically the population of Moina is always constituted of two groups of moderate amount. Pond No. 3 seems to belong to this type from the view of its size composition.

The third type differs essentially from the above mentioned two types. In this case, the population is constituted of two different species of Cladocera. In this investigation the population of cladocera of the pond No. 4 was constituted of both

*Moina macrocopa* and *Moina weismanni*, and the latter showed its peak of abundance later than the former.

(ii) In the fish ponds parthenogenetic generation of Moina does not last for a long time, because the fecundity of parthenogenetic females in all ponds decrease in a short time. For instance, Moina in the Pond No. 4 showed its best condition on the 21st, namely summer eggs in their brood chamber were fifteen, but three days after they dropped to four, and the percentage of the ephippial females to the parthenogenetic ones increased.

(5) An examination on the problem of additional fertilizer.

Additional fertilizing is widely used for practical method to increase the Moina in the ponds, whenever the population decrease takes place. Regarding this problem little is known, but if it is effective really, this treatment means to supply the deficiency of food of Moina.

Ammonium sulphate and boiled wheat flour have been used by many fish farmers. But as mentioned above, the changing of the generations from parthenogenetic to bisexual, is the chief cause for the population decrease of Moina, and when it occurs, the increase of the percentage of ephippial females may invariably be observed. Accordingly, experiments were intended to make clear the possibility of changing of the ephippial females to parthenogenetic ones by the treatment of additional fertilizing.

A great number of ephippial females of Moina were collected from the aquaria in the laboratory, and their gonads were examined microscopically. Their gonads are not uniform in shape and stages of the development of winter egg were observed as is described in Fig. 5. The direct effect of the additional fertilizing was examined in the following method. Ephippial females were put individually into a vessel fertilized with organic fertilizer. In this experiment dry chick dung was used.

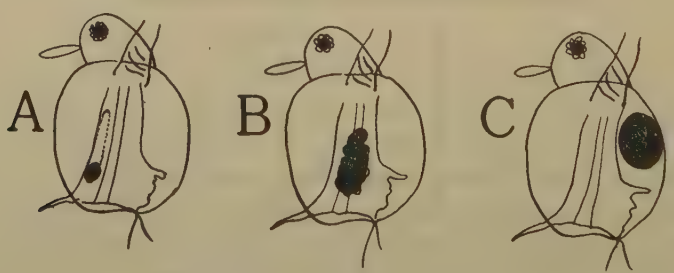


Fig. 5. Three stages of winter egg.

A : The origin of the winter egg. It is situated to the left side of the intestine.
B : Middle stage of the growing winter egg. It is on the intestine.
C : Winter egg in the brood chamber, ephippium is already completed.

As is shown in Fig. 6 the effect of the additional fertilizer is related to the stage of the maturation process of the sexual egg. For example, when the eggs developed in the length over the value of thirty percent of body length, the ephippial mother could not be changed to parthenogenetic ones. She continues the formation of sexual

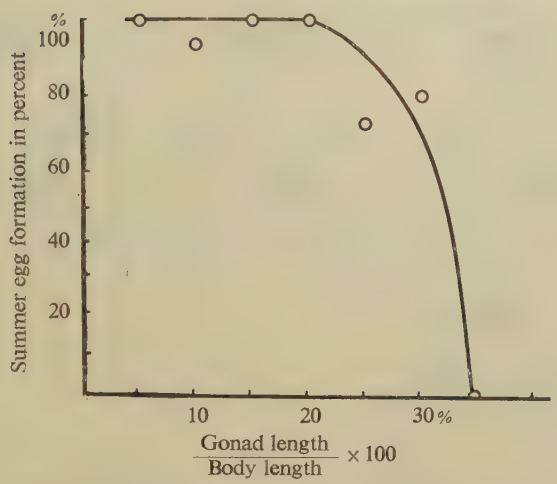


Fig. 6. Effect of the additional fertilizer to the winter egg forming Moina.

egg and completes it in the ephippium, even if it was put in the well fertilized solution. On the contrary, opposite result is expected when the gonad length is less than thirty percent of its body length.

3. Observations on the stock of the winter eggs of Moina in the pond, with a special reference to its decreasing.

It was already mentioned that the winter egg of Moina has a long sleeping at the bottom of the pond until the next favorable season comes. At Gunma Prefecture in the Kanto district the sleeping period is about eleven months from June to April of the following year. It is doubtful whether the pond bottom is a suitable place to keep the eggs for a long time, because it is ordinarily covered with organic silt in some depth and it bears many inadequate conditions to keep the eggs.

We have little informations with the amount of winter eggs in the ponds and their decrease in a year. In order to clarify these problems the following points were observed.

(1) Types of winter egg formation.

A great many samples were collected, not only from many fish ponds but also from small outdoor and indoor aquaria.

Morphological study of these samples were performed. Besides these morphological study, observation with the procedure of winter egg formation of Moina in nature and in the laboratory were done. And it may be given as a conclusion that there are two types of winter egg formation, the one is ordinarily seen in fish ponds and the other is observed usually in a small aquaria in the laboratory. They have not only the different size of the produced egg but also the different frequency of production. In fish ponds the same individual produces repeatedly many winter eggs, but in a small vessel of the laboratory winter egg formation takes place only one time in its generation. These two types are explained in Fig. 7.

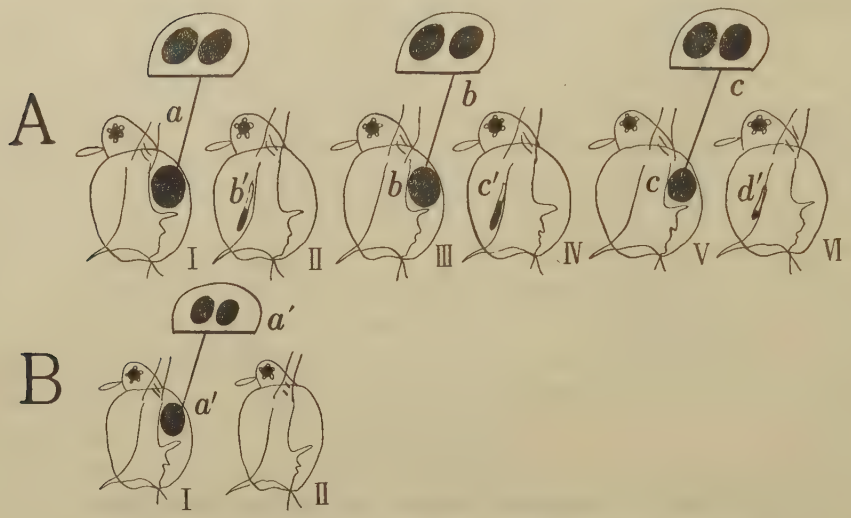


Fig. 7. Explanation of two types of winter egg formation in Moina.

A : This type is found commonly in the carp-culture ponds. The same mother produces repeatedly many winter eggs.
In II, IV, VI she shows the origin of the winter egg just after she produced a, b and c.

B : This type is observed in a small vessel in the laboratory. Winter egg formation is seen once in her generation.
Winter eggs produced in A type are larger than that in B.

(2) Non-existence of pseudosexual egg in Moina.

It was reported already that the pseudosexual eggs exist in some species of Daphnidae. And it is well known that there is a remarkable difference between the pseudosexual egg and the true sexual egg, in spite of their same shapes, namely, the former has a diploid and the latter has a haploid chromosome. Following experiments were performed to prove the non-existence of pseudosexual egg in Moina.

25 ephippial females with well developed gonad were put in a small vessel, and vigorous males were poured into this vessel. Females used are all 1200–1300 $\mu$ in length and their gonad length are also over 30% of their body length. Males are 800–900 $\mu$ in length. Following five combinations are prepared.

♀ : ♂ 25 : 0, 25 : 5, 25 : 10, 25 : 15, 25 : 25

Obtained results were shown in Fig. 8.

True winter eggs are not produced in any case in the vessel which is free from males. Instead of true winter eggs there exist many empty ephippia. Accordingly, winter eggs of Moina are always fertilized eggs.

(3) Distribution of the winter eggs in the ponds.

The amount of the first larvae in the propagation of Moina in the pond is decided by the amount of the winter eggs produced at late May of the previous year and its survival rate during eleven months, but our knowledge regarding the distribution and the amount of the winter eggs of Moina in the ponds are too me-

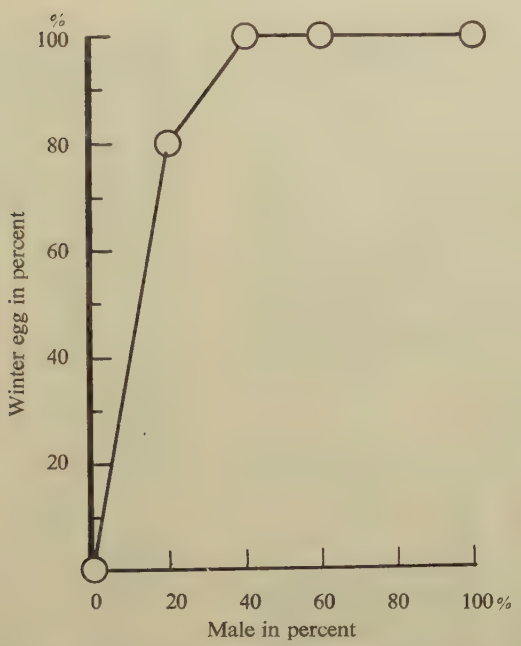


Fig. 8. Relation between sex ratio and the produced sexual eggs (%).

Females that are free from males do not produce the true sexual eggs. They produce only empty ephippia. This experiment shows non-existence of pseudo-sexual eggs in Moina.

agre up to now.

Three ponds in two prefectures far from each other were selected, two of them are located in Gunma and one is in Yamaguchi. Investigation was performed December 1956 and March 1957. Survival rate was calculated from the difference between the estimated early stock and the real stock at present. Obtained details were recorded in Table 2 and Fig. 9.

Results obtained from this investigation are as follows:

(i) The distributions of the winter eggs of Moina in three ponds are not uniform in their pattern, and it was difficult to find a standard pattern even if in two neighbouring ponds of Gunma Prefecture.

(ii) The amount of last survivor is estimated as 4,000–50,000/$m^2$ and decuple variation existed between these three ponds.

(iii) Survival rate ranged between 2–8 percent.

(iv) Distribution of bottom silt is related to the distribution of winter eggs in the Pond No. 4 of Gunma Prefecture.

(4) Survival curve for the winter eggs of Moina in the pond.

The factors related to the survival rate of the winter eggs will be divided into two categolies, namely biological and physicochemical. Data in Fig. 9 and Table 2 indicate mean value of last survivor, but the details in the course of decreasing were unknown. Hence, in 1959 an attempt was made to make clear the decreasing

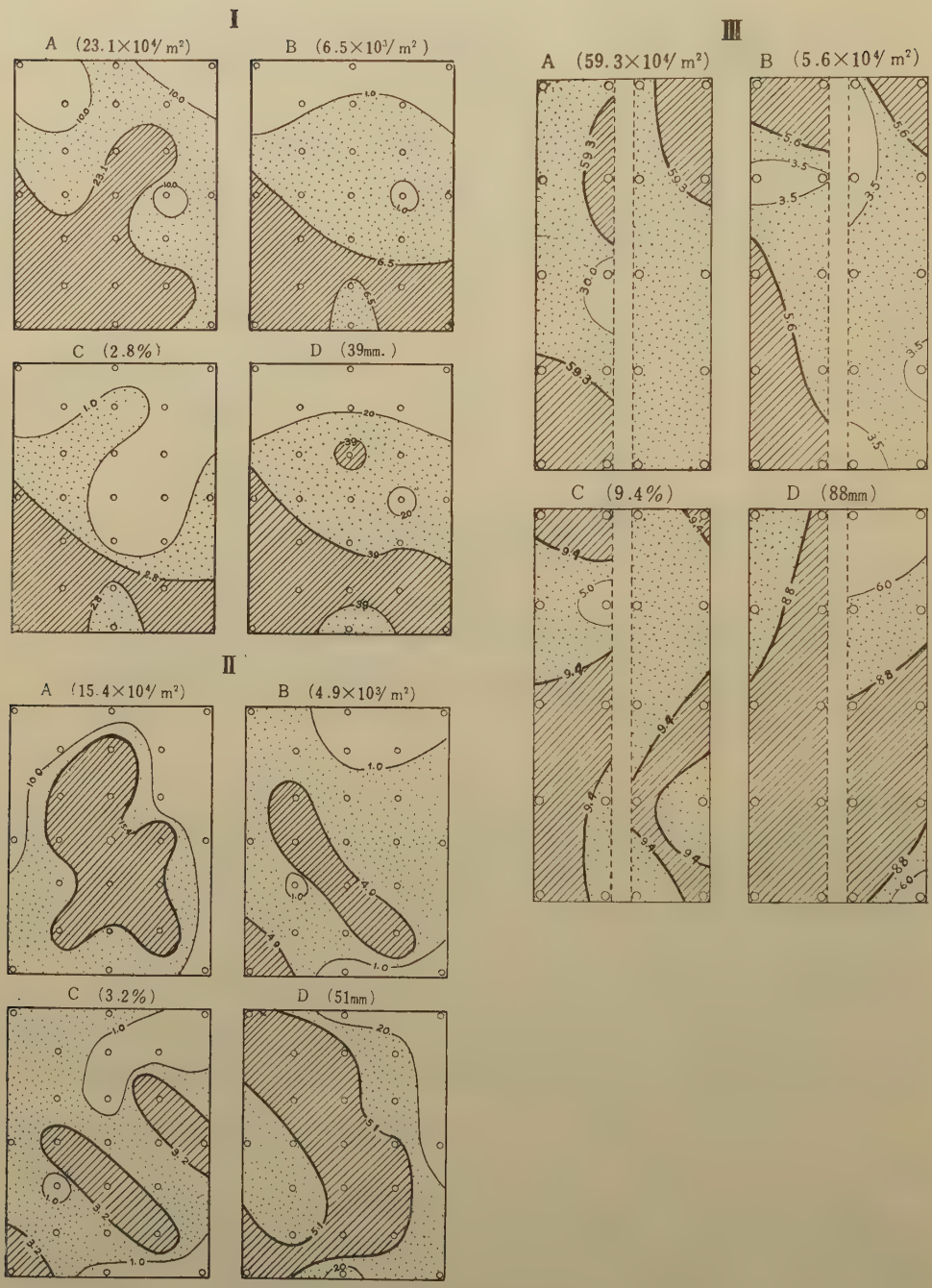


Fig. 9. Maps showing the variation in the distribution of winter eggs and mud.

I, II and III: ponds Nos. 3 and 4 in Gunma and the pond in Yamaguchi respectively.

A) estimated total number of winter eggs produced (/m²), B) number of winter eggs at the present investigation (/m²), C) survival rate of winter eggs (B/A × 100) (%), D) mud depth (mm.). Average values for each item are given in brackets. Areas showing higher values than average are crosslined, and open circles represent the sampling stations.

Table 2. Number of winter eggs obtained in various ponds.

| Pond | Number of sampling stations | Estimated number of winter eggs produced /m² | | Number of winter eggs in the present investigation /m² | | Survival rate of winter eggs % | | Mud depth mm. | |
|---|---|---|---|---|---|---|---|---|---|
| | | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| Gunma, No. 3 | 23 | 26,900–838,500 | 231,300 | 0–48,000 | 6,500 (4,900) | 0–8.5 | 2.8(2.1) | 0–96 | 39 |
| 〃 , No. 4 | 23 | 13,300–410,400 | 154,300 | 0–35,000 | 4,900 (3,600) | 0–12.1 | 3.2(2.4) | 0–120 | 51 |
| Yamaguchi | 20 | 260,600–1,210,500 | 593,300 | 28,100–146,900 | 56,000(47,000) | 3.7–18.3 | 9.4(7.9) | 50–120 | 88 |
| Note<br>Gunma No. 5 | 14 | 0—209,500 | 52,300 | 0—9,900 | 1,400(1,000) | 0—6.3 | 2.3(1.9) | 15—90 | 30 |

Figures in brackets are the corrected values determined after examining the hatchability of the eggs. Hatchabilities were 75% in Gunma samples and 84% in Yamaguchi ones.

process of the eggs in the ponds. Collections of samples were performed in the ponds of Gunma, Tokyo and Hiroshima Prefecture. Survival curve was shown in Fig. 10.

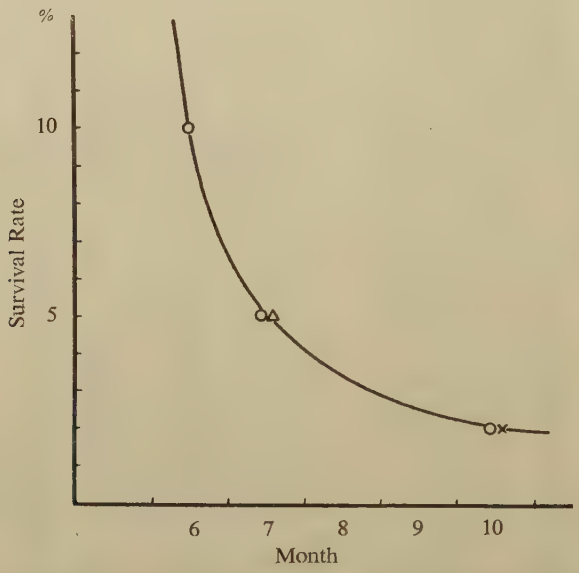


Fig. 10. Survivorship curve for the Winter Eggs of Moina in the carp-culture ponds.
○ Gunma △ Hiroshima × Tokyo

In Fig. 10, it is clear that the curve drops sharply in a month just after the winter eggs were produced in the ponds, then it goes gently sloping and after late October it seems to continue in the same level, because the value of late October of 1959 agrees with the value of 1956. Conceivable factors related to the survival of the winter eggs are as follows.

i) Percentage of fertilization
As mentioned above, copulation or fertilization is the first requisite factor to produce the winter eggs.

ii) Percentage of imperfect ephippia.
When the winter eggs are not closed perfectly in the ephippia, they fall out easily and only empty ephippia are found there.

iii) Bacteria attack will be the most innocuous factor.

iv) Fungus parasite
Investigations on this problem are explained in the next chapter.

(5) Sleeping of the winter eggs of Moina in the ponds.

Sleeping is the most characteristic and noteworthy phenomenon in the ecological and physiological studies with the Cladocera, because the keys to solve many problems with the Cladocera are expected to exist in the explanations of sleeping of the winter eggs. For example, the cycle of alternation of their generations and the survival rate of the winter eggs are deeply related to this phenomenon.

Sleeping of the winter eggs is generally accepted as a kind of the delayed germination, and if it is true, the sleeping control factors will be inherent in the winter eggs themselves, not in the environmental conditions, and the differences of the number of cycles of alternation of generations will be caused by these inherent differences. For instance, if Moina shows three types of cycle, namely, monocyclic, dicyclic and polycyclic, these differences indicate the existence of three different winter eggs of Moina each having different sleeping times.

From the point of this view, the monocyclic aspect of Moina in the carp-culture ponds of Gunma Prefecture could be explained as follows: The eggs produced in late May are not mature enough to begin their development immediately and their maturation proceeds slowly with the elapsed time until their hatching takes place on early May of the following year.

But there is another opinion with this sleeping of the winter eggs of the Cladocera, this is, an explanation based on environmental factors of the places where the winter eggs are preserved for a long time.

Apparently there is a discrepancy in interpretation between these two opinions. One is chiefly based on the biological inherent natures of the winter eggs themselves, on the other hand, the other gives priority to the environmental factors.

Many experiments were performed in the laboratory with the hatching of the winter eggs of Moina collected from many carp-culture ponds in every season round a year, but their results were always denying the hypothesis that the sleeping of the winter eggs of Moina is a kind of delayed germination, because in all cases of these experiments their hatching rate always indicated a fairly high value, even if the material was collected on late May just after they were produced.

Winter eggs of Moina that were obtained in aquaria of the laboratory under various conditions were also tested, but they showed always the same results as in nature.

On the contrary, following fact was experimentaly proved that when the winter eggs of Moina were buried into the deposit of the Moina culture tank, the hatching of them did not take place, even if other conditions were favorable to their development. Bottom silt of the carp-culture ponds and its filtrate are also similarly effective in inhibiting their development.

Furthermore, when those controlled winter eggs were transferred into tap water their hatching rate showed the same high value as those not controlled by the above mentioned substances, so it is right to interprete the sleeping of the winter eggs of Moina at least as a phenomenon to be that their development is inhibited by environmental factors.

Both in Moina culture tank and in carp-culture ponds, a great amount of organic matter are used as a fertilizer and the deposit or bottom silt are more or less polluted with their decomposition added by the disassimilation of organisms. Rearly, they show always a positive ammoniac reaction to the Nessler reagent.

It is well known that ammonia and ammonium compounds have a toxic action to aquatic organisms, and as above mentioned the sleeping of the winter eggs of

Moina is a kind of compelled inhibition of the development, then the following experiments were done to make clear the relations between them.

Fifty winter eggs of Moina were sealed into ampoules (15 cc in volume) which have different concentrations of ammonia and ammonium compounds solution. These ampoules were kept at 25°C, and they were tested at intervals of a week.

Development control power of ammonia and ammonium salts solutions were decided by the percentages of the number of the deformed winter eggs during preservation, not by the hatching rate, because as the first prerequisite the size immutability is demanded for the sleeping of the winter eggs.

And it is also well known that pH value is very important, but in these experiments the solutions $NH_4Cl$, $NH_4CO_3$ and $(NH_4)_2SO_4$, were not regulated, accordingly their pH values show less than 7.

Experiments with solutions of ammonium salts showed the following results that all winter eggs kept therein were deformed, even if their concentrations of ammonium ion are more than 100 p.p.m.(N). So it will be said that the sleeping of the winter eggs of Moina can not be attained solely by application of ammonium ion. On the contrary, experiments in ammonia water show some interesting results. As showed in Fig. 11 ammonia water over 10 p.p.m.(N) apparently showed to have control power to the development of the eggs, and according with the decrease of the amount of ammonia their control power dropped. Here, it was also experimentaly proved that high pH value, for example, pH 9–10 of the $H_3BO_3$, KCl and NaOH buffer solutions could not inhibit the development of the eggs, and as above mentioned the amount of not ionized ammonia are effective more than ten times of ionized ammonia. So it is conceivable that free ammonia plays an important role for the sleeping of the winter eggs of Moina.

But such amount of free ammonia of 10 p.p.m. of ammonia water could not be expected in nature, even if the amount of ammonium ion indicates the same value. Furthermore, it is unforgetable that the survival rate of the winter eggs of Moina in the carp-culture ponds are usually only 2–3 percent and the sleeping phenomenon is rather imperfect at the bottom of the ponds. Besides ammonia and ammonium ion as the sleeping control environmental factors the following ones are expected, namely water temperature and the amount of the dissolved oxygen. For example, low value of these factors inhibit the development of the eggs, but on the contrary, both high temperature and plenty amount of dissolved oxygen activate the development.

In Fig. 11 the long time preservation of the eggs in 10 p.p.m. ammonia water indicates the decrease of hatchability, and so it is desirable to use weak ammonia water to preserve the winter eggs for a long time.

From this point of view, bottom silt of the ponds were again tested, as a practical preserving method of the eggs, because if it is alkaline, the organic polluted mud always contains more or less ammonia and the consumption of the dissolved oxygen is always expected there.

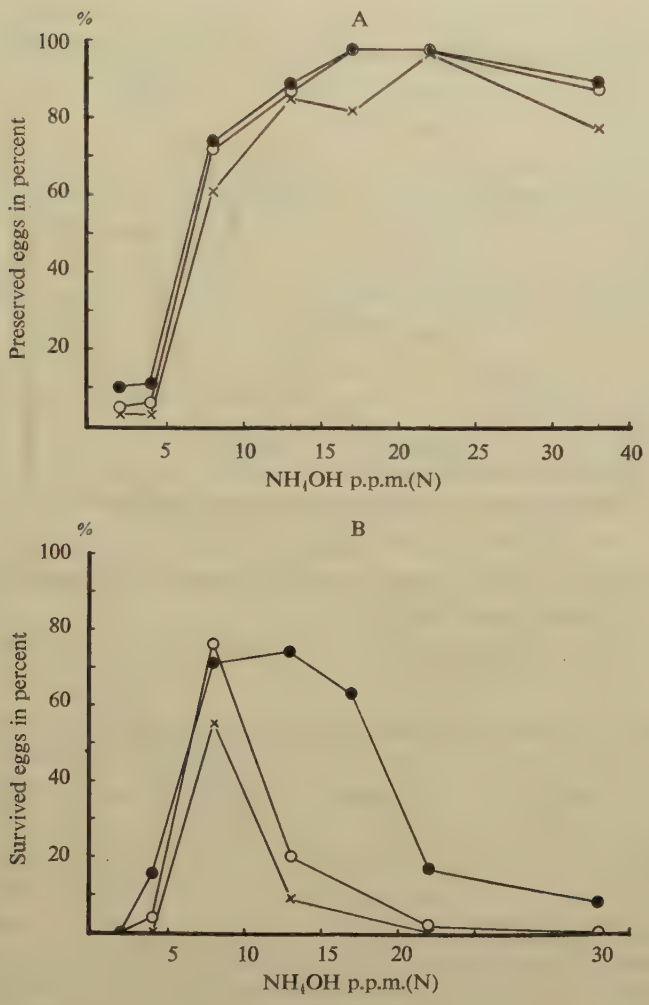


Fig. 11.

A : Relation between the concentration of ammonia and its effect to prevent the development of the Winter Eggs.

B : Relation between the survival rate and the concentration of ammonia.

—●— Preserved in 10 days
—○— // 20 //
—×— // 30 //

One example of many experiments is shown in Fig. 12.

For the long time preservation of the winter eggs of Moina, the amount of organic matter of the bottom silt is important, because if it is in excess, the putrefaction usually takes place, and the death of eggs happens.

Practically, satisfactory results are always obtained when the winter eggs are preserved in the bottom silt of the ponds, but here it is necessary that the mud must be filtrated with tap water several times. For instance in Fig. 13 the survival rate and hatchability of the eggs preserved in this method is shown.

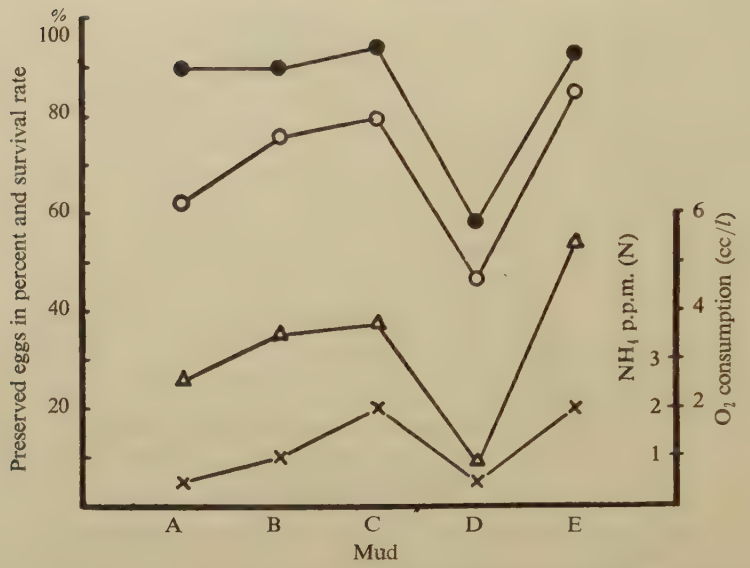


Fig. 12. Relation between the sleeping of the Winter Eggs and the bottom silt of the carp-culture ponds. (January 1958)

—●— Preservation rate —○— Survival rate —△— $O_2$
—×— $NH_4$ p.p.m. (N)

Table 3 Effect of the bottom silt to the sleeping of the Winter eggs of Moina

| Mud | Pond | Date | $O_2$ consumption cc/l 9°C 24 hrs. | $NH_4$(N) | Preservation rate % | Survival rate % |
|---|---|---|---|---|---|---|
| A | Gunma No. 5 | 1956. 5 | 2. 64 | 1> | 89. 5 | 62. 3 |
| B | 〃 | 1956. 12 | 3. 47 | 1 | 89. 6 | 75. 5 |
| C | Shizuoka Eel-culture Pond | 1958. 1 | 3. 76 | 2 | 94. 0 | 79. 4 |
| D | Yamaguchi Carp-culture Pond | 1957. 2 | 0. 88 | 1> | 57. 7 | 46. 3 |
| E | Laboratory | 1957. 10 | 5. 39 | 2 | 92. 2 | 84. 3 |

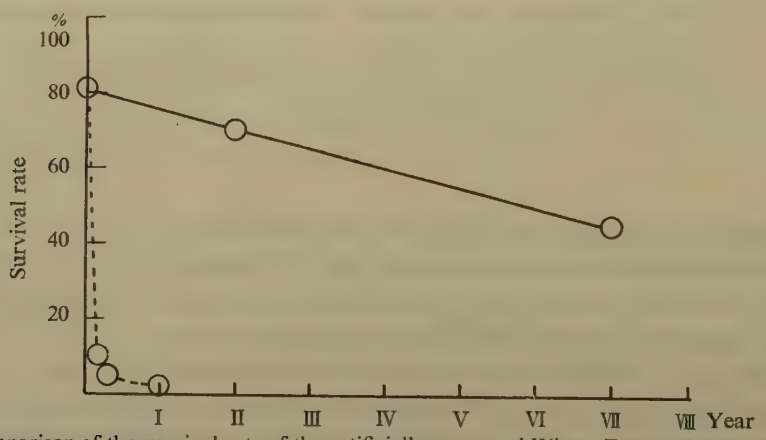


Fig. 13. Comparison of the survival rate of the artificially preserved Winter Eggs and that in nature.
····· Eggs in nature —— Artificially preserved Eggs

Abnormal eggs of Moina

It is already mentioned that in Moina pseudosexual eggs do not exist, but the existence of abnormal eggs is one of the most characteristic and noteworthy phenomena.

Specimens used in this experiments are always microscopically examined with their size and shape, but it is impossible to know whether they are normal or abnormal by the morphological examination only, because occassionally their hatching rate shows very small value in spite of their normal shape. These abnormal winter eggs are only distinguished by the difference of their viscosity from the normal ones, the former is less than the latter.

Existence of these abnormal eggs is one of the most important causes of the seemingly unreasonable results of the increase of the hatchability of the eggs at the later season. If the number of these abnormal eggs is much at earlier season than at later season, and they decay to empty ephippia with the elapsed time, the hatchability of the tested winter eggs indicate high value. So the fluctuation of hatchability must be necessarily examined with the reference of constitution of the eggs.

Other preservation methods of the winter eggs were also examined, but good

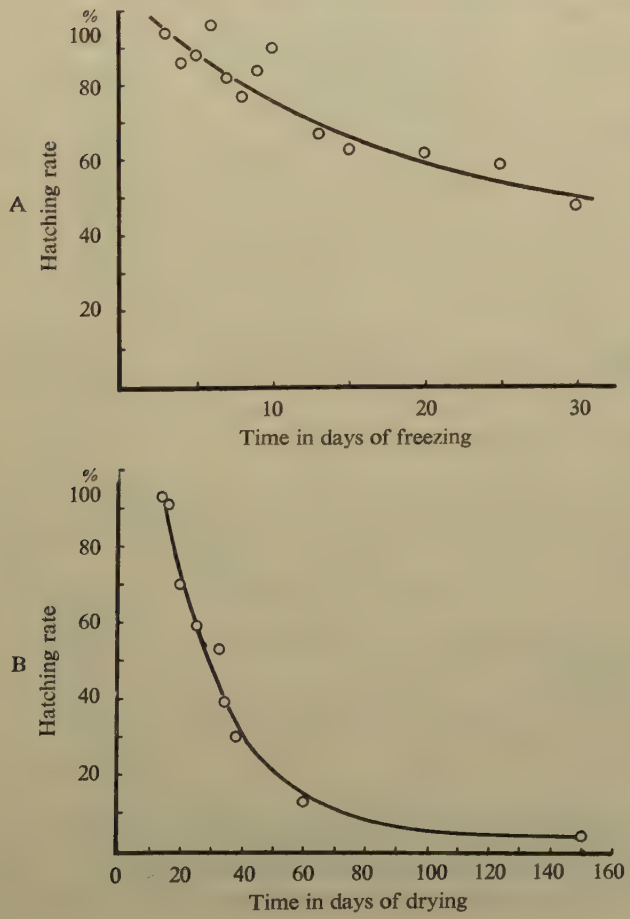


Fig. 14. Preservation of the Winter Eggs of Moina by freezing and drying.

results were not obtained than in the case of the methods of above mentioned bottom silt. In Fig. 14, the results of the two methods, freezing and drying are shown.

4. A parasitic fungus, *Lagenidium* sp.

In early June, 1959, fungus parasited winter eggs were obtained in carp-culture ponds of Gunma Prefecture. This was the first collection during this ten years observation with the winter eggs of Moina in many carp-culture ponds in Japan. These specimens were observed by microscope in the Laboratory of the University of Tokyo, and its asexual reproduction was partially clarified as follows. Under aerobic conditions the parasite fungus shows interesting figures as described in Fig. 15 and Plate 1 and 2. This fungus was identified to belong to *Lagenidium* sp. by the kind aid of Mrs. SUNAYAMA of Tôhô University.

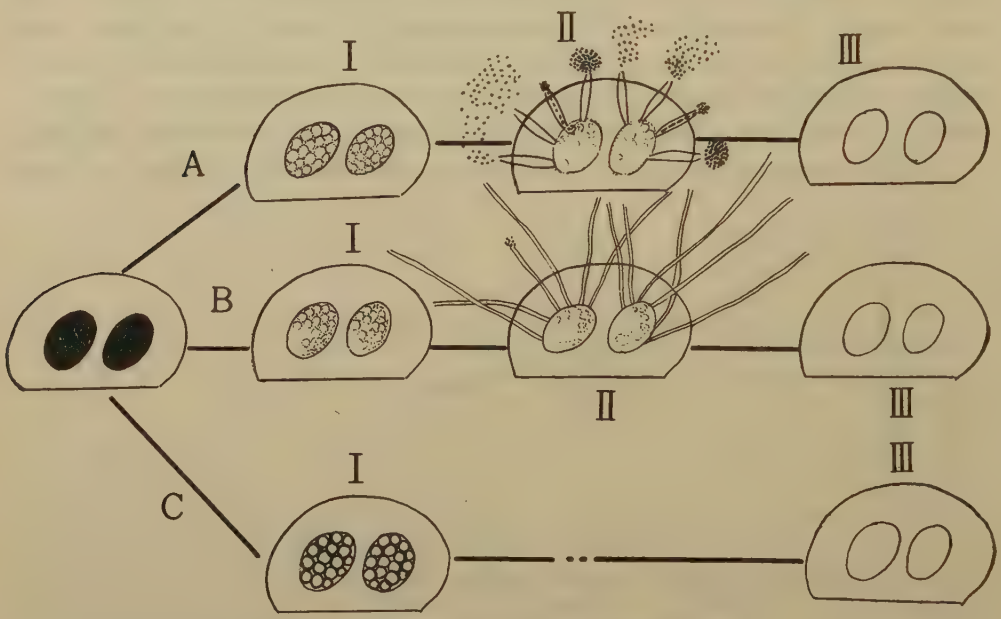


Fig. 15. Explanation of the asexual reproduction of a Lagenidium sp.
Three types were observed.
A I shows the deformed Winter Eggs infected by the zoospores of a Lagenidium sp.
A II shows many discharge tubes and liberation of the zoospores. These zoospores attack again other healthy Winter Eggs.
A III When zoospores liberated out, there remains a empty ephippium.
B I is like to A I.
B II Many long and narrow discharge tubes are formed, but zoospore can not be found. When the zoospores are liberated, they are very few.
B III B II changes to a empty ephippium.
C is different from A and B. It makes resting spores with thick membrane. These spores do not change to zoospores in a short period.

Under the favorable water temperature, 20–30°C, many infection experiments were performed and the cycle of their asexual reproduction were observed. This fungus is holocarpic and when the zoospores entered into the healthy winter eggs

of Moina, the formation of discharge tubes were observed, then many zoospores come out through the tube in a single file, and encysted just outside of the mouth of this discharge tube. The length of tubes was 100–700$\mu$, sometimes over 1500$\mu$, and its width was 10–25$\mu$.

Soon after they became encysted, they formed a spherical aggregation, (80–120$\mu$ in diameter), containing 20–200 zoorpores. These spherical aggregation grew rapidly. After an active rotating movement, the zoospores swam out into the water almost as the same time, not separately. Zoospores are bean shaped (10–20$\mu$ in length) and biciliate.

These escaped spores successively attack the healthy winter eggs and there remain many empty ephippia.

Concerning the distribution of this fungus little is known, but it is supposed that it will take wide distribution in the carp-culture ponds in Japan, because they were found in the ponds of Hiroshima Prefecture of the Chugoku district in July 1959.

The infection of this fungus can be apparently observed in nature when they form discharge tubes in the winter eggs of Moina, but at the earlier stage it is rather difficult to distinguish the parasite eggs from normal one.

Percentage of the infected eggs observed in nature shows less than 10%, but in laboratory it shows more than 50%. Here, the bottom silt again plays an interesting role, namely, the fungus in the winter eggs is preserved in it for a long time, and it developes when the eggs were set under aerobic condition.

It was experimentally proved that when one infected winter eggs is mixed in the healthy ones, their hatchability remarkably decreases, occasionally no hatched larvae were observed, even if 99% of the winter eggs are healthy ones.

Infection experiments by this fungus to the winter eggs of Moina have an

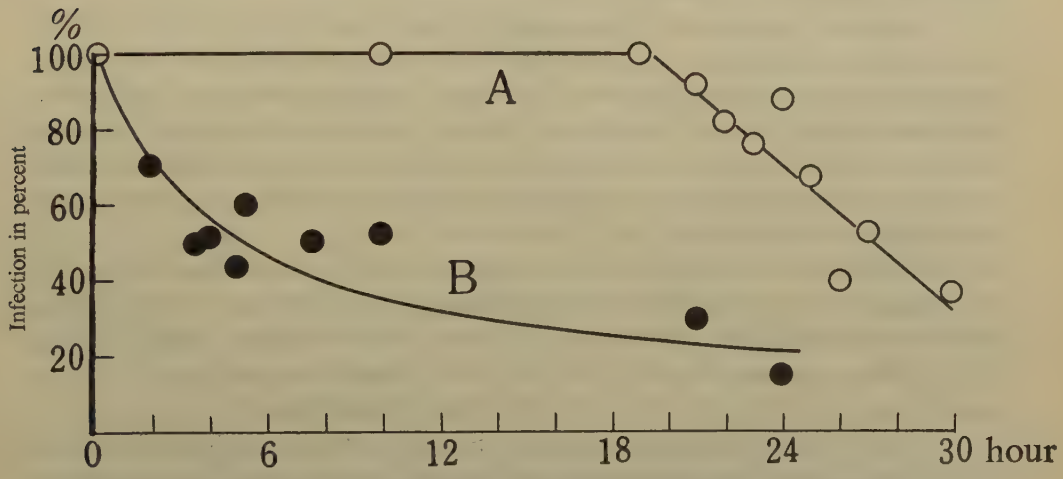


Fig. 16. Relation between the stage of the development of the winter eggs and their infection rate. The development of A goes slowly than B. Using these infection experiments the activity of the winter eggs can be tested in a short time.

unexpected availability to know the hatchability of the eggs in a short time. In order to know the hatching rate of the winter eggs in tap water several days are needed but the cycle of the asexual reproduction of this fungus is observed in thirty hours.

It was experimentally known that the infection rate of this fungus to the winter eggs is related to the activity of the eggs. For instance as shown in Fig. 16, the infection rate shows different figures in different materials and in different developmental stages of the same material.

Winter eggs that have different exposure times in tap water were infected artificially with this fungus zoospores. In sample A the infection rate shows the same value of 100% till 19 hours exposure, but at 20 hours it begins to drop almost in rectilineal form till 28% at 30 hours exposure. Accordingly by the test of this infection experiment, the winter eggs of sample B is more active than those of sample A, in other words, from the point of view of quality, the sample B is better than sample A.

## (IV) DISCUSSION

In almost all fish farms in Japan organic substances are used as fertilizer in Moina culture, such as cattle manure, barnyard manure and soy lees, and they furnish plenty of foods for Moina, containing bacteria, protozoa and unicellular phytoplankton.

Among these foods for Moina, the bacterial growth will serve as the principal ones. Concerning the culture methods of water fleas many works have been reported, for instance, BANTA, CHIPMAN, PRATT, IMAI and SATO, MATSUDAIRA, MURACHI, FUJITA and many other investigators reported on this problem with special references to the food organisms.

Population growth and its fluctuations of water fleas have been taken as one of the most interesting problems in ecological and physiological studies of zooplankton in fresh water, and the works with these problems have been reported by many investigators, for example, PRATT, TERAO, FRANK and SUGINOME. But our knowledge with the growth form of population of Moina in large carp-culture ponds are meagre, notwithstanding the history of propagation of this water flea goes back to old times in this country.

In this investigation efforts have been made to obtain the standard feature for the propagation of Moina, and special attentions have been directed to the qualitative and quantitative fluctuations of population. In the culture of water fleas not only in laboratory but also in nature, the maintenance of the parthenogenetic reproduction during long time is the most ideal and desirable form.

As long as the theoretical population growth of Moina holds good, a great amount of it is expected in the ponds. For instance, if this water flea matures by four days from its hatching and she produces ten larvae every day and ten times in the generation, the following tremendous increase of the number of individuals is theoretically expected.

| DAY | 1 | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|---|
| Number of Individuals | 1 | 11 | 360 | 12. 900 | 478. 000 | 19. 020. 000 | 767. 170. 000 |

But in this calculation the following conditions are necessary:

1) Population is constituted with females;
2) productivity of mother shows always high level: 10 summer eggs are produced in her brood chamber at one time;
3) winter egg formation does not take place;
4) difference between the amount of young and mother is rather small.

But the analysis of the population growth form of Moina in the carp-culture ponds indicates that those conditions cannot be expected to exist in any way in the ponds. And so it is necessary to introduce a new technical treatment to solve these problems. From this point of view the amount of the winter eggs in the ponds must be carefully examined, because it is directly related to the amount of the first population of Moina. Furthermore, it will have more important meaning with the consideration of the fact that the first larvae hatched from the winter eggs produce summer eggs during their generation and they do not produce winter eggs.

Amount of the survived winter eggs during a year shows about ten times difference in the investigated ponds, from $5 \times 10^4$ to $4 \times 10^3/m^2$, and this difference may be significant when parthenogenetic reproduction does not continue for a long time.

Additional fertilizer and the seeding of winter eggs will be available techniques. As a practical method to preserve winter eggs as "seeds", the bottom silt in the ponds is useful. Caution must be paid to the parasitic fungus and its infected winter eggs, but details are remained in further investigation.

## (V) SUMMARY

1) *Moina Macrocopa* STRAUS appears in the carp-culture ponds in May, but disappears during following eleven months, from June to April of the following year remaining dormant in the form of winter eggs kept in the bottom silt.

2) Population growth of Moina in the ponds was investigated, and it is classified into three types. The first is characteristic by the sharp decline of the parthenogenetic reproduction which then shifts to the bisexual phase. In the second type the growth shows a fluctuation taking a wave shape, and in this type considerable population growth is expected. The third differs fundamentally from those above mentioned. In this case two distinct species of Moina come into being showing their growth peaks at different times.

3) Community of Moina even in the neighboring ponds shows different patterns, and there its great fluctuations are met with occasionally.

4) The amount of the winter eggs of Moina was from $6 \times 10^5$ to $5 \times 10^4/m^2$ on the bottom of the pond, and their survival rate were estimated as 2–3%.

5) 'Sleeping' of the winter eggs of Moina is explained as that it is a kind of

inhibition of development enforced by environmental factors, and is not a delayed germination attributable to their own inherent character.

6) Ammonia is undoubtedly effectively related with the sleeping, but the best method to preserve sleeping eggs for a long time is the use of bottom silt.

7) Two types of winter egg formation exist in nature, but in the carp-culture ponds the type of successive formation of the winter eggs by the same mother is common, and this is the reason why there exists such a great amount of winter eggs in the ponds.

8) Non existence of the pseudosexual eggs was experimentally proved.

9) A sexual reproduction of a parasitic fungus, *Lagenidium* sp., was proved clearly by the infection experiments of its zoospores to the healthy winter eggs of Moina. This fungus is available to know the activity of the egg development.

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## EXPLANATION OF PLATES 1–2.

Successive liberation of zoospores of the six different discharge tubes of Lagenidium sp. at 25°C.

Fig. 1, 2, 3, 4. Process of the forming of the spherical aggregation of zoospores and their liberation of the discharge tube a.

Fig. 5, 6, 7, 8, 9, 10. Process of the encystment, and the liberation of the newly formed discharge tube b. Enlargement of the spherical aggregation of zoospores is observed.

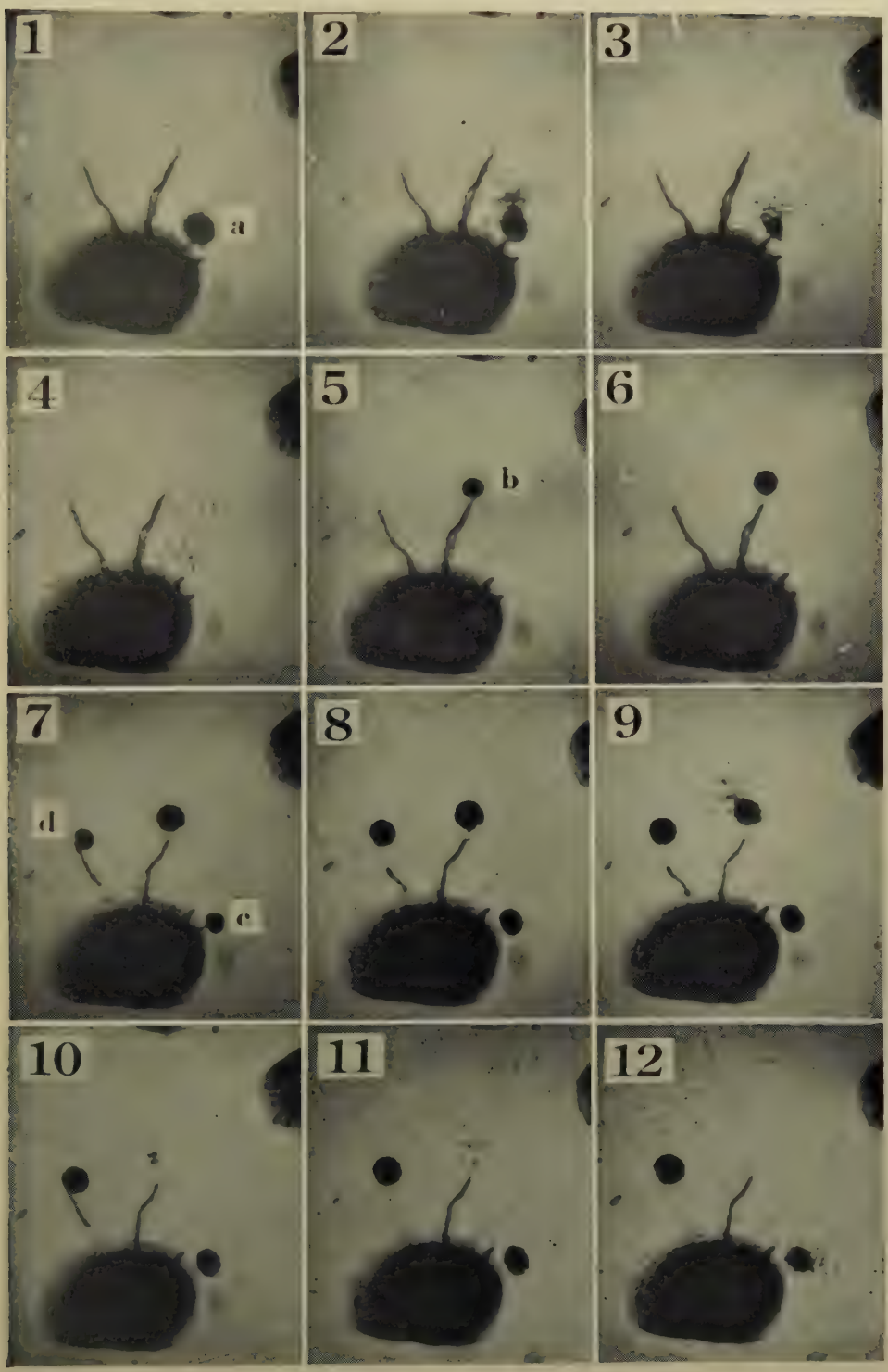
Fig. 7, 8, 9, 10, 11, 12, 13. The same process of the third tube c.

Fig. 7, 8, 9, 10, 11, 12, 13, 14, 15. Ditto process of the fourth discharge tube d. In Fig. 9, 10 indicate the escape spore and the empty tube.

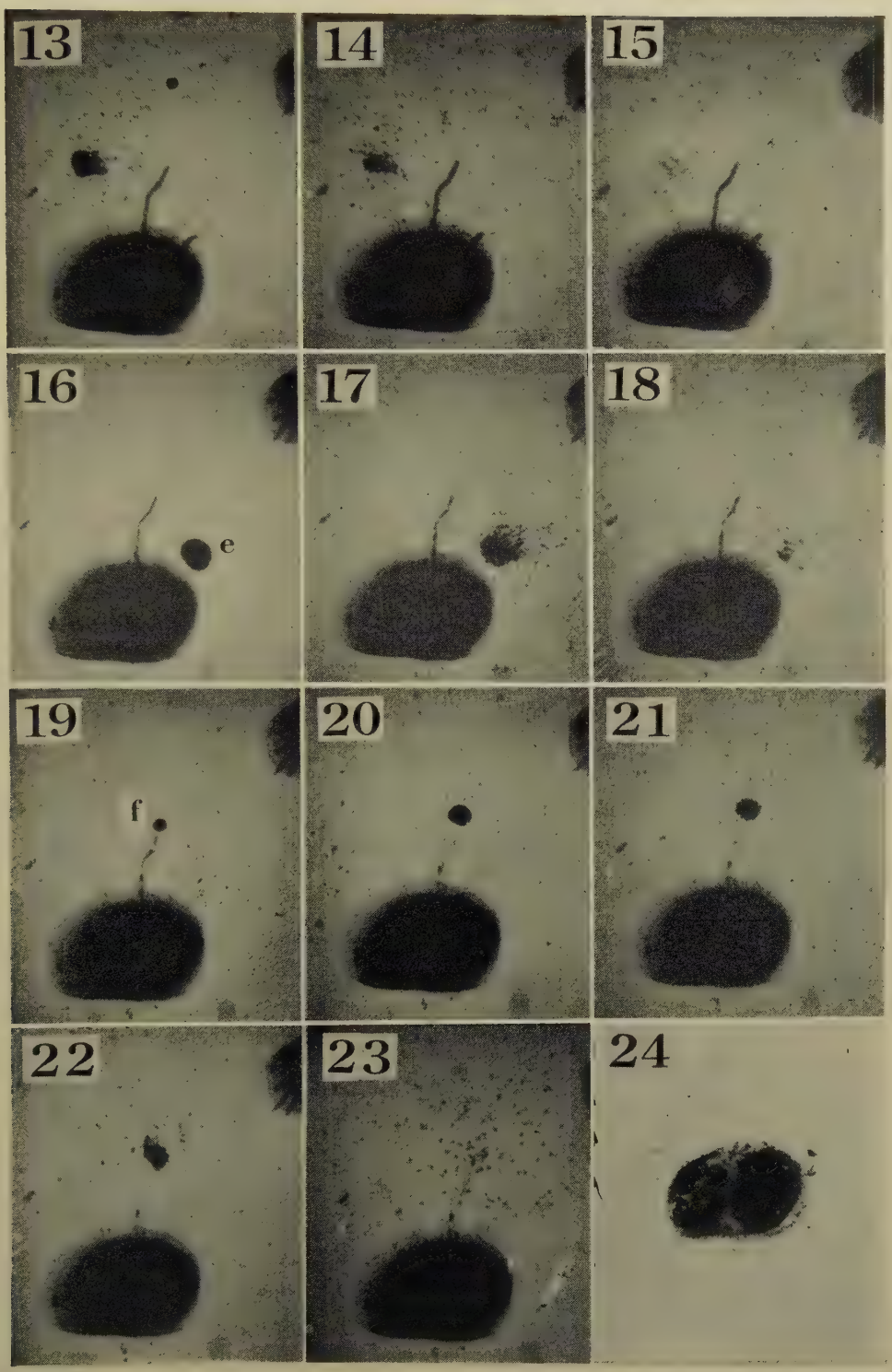
Fig. 16, 17, 18. The same process in the fifth discharge tube e. Liberation of zoospores takes place all at once.

Fig. 19–23. The same process in the sixth discharge tube f.

Fig. 24. Showing the winter eggs packed with resting spores with thick membrane.




MURAKAMI : Winter Eggs of Moina




MURAKAMI : Winter Eggs of Moina

# Some Considerations on Diving of Whales

Yasutaro Ishikawa

*Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Tables 1–4)

## INTRODUCTION

It has long been a recognized fact that whales can dive much longer and much deeper than other diving pulmonate animals. Especially, the sperm whale and the bottle-nosed whale are known to endure most prolonged and deepest submergence. Some experimental evidences and theories have been presented by a number of authors to elucidate the long apnoea of diving animals. It seems however, very interesting to the present author that the sperm whale and the bottle-nosed whale, both being the champions of all pulmonate divers, possess within their body a large quantity of wax, instead of usual fat. Moreover, the sperm whale has an enormous special organ, *spermaceti*, which is situated over the skull, the content of which is largely constituted of waxes. The physiological function of this organ however, has not yet been clarified. No other animals are known to possess *spermaceti* or any organ similar to it, except that the bottle-nosed whale has a small organ which is probably homologous to the *spermaceti*. As for the presence of large quantities of wax, similar instance is not known in any other animal than the two species named. Even in other whales, usual glycerides constitute the major part of the fatty substances together with a small amount of sterols and other unsaponified matters, as it is the case in other animals.

The problems to be considered in this article, are (1) what factors enable the whales particularly the sperm whale to endure apnoea so much longer than other animals and (2) how they can avoid getting the caison sickness, which may attack them when they emerge from a deep dive. Now we are to survey briefly the duration and the depth which whales can stand and reach.

## DURATION AND DEPTH

Duration—. Table 1 shows the duration of dives of various mammals and birds which can anyhow endure diving apnoea. From this table, we can see that no other animals than the whale endure longer dives than 15 minutes. Only the seal and the manatee are tolerable for 15 minutes or little longer diving apnoea. Though many observations have been made on the duration of diving of whales, few informations are reliable. Descriptions based on the conversation of gunners are to be taken with some caution, because the estimations which are made by the gunners in

Table 1. Duration of Dives of Mammals and Birds (after Irving and Scholander)

| Animal | Time | Authority | |
|---|---|---|---|
| * Pigeon | 1 min | Bohr | 1897 |
| * Hen | 3 〃 | 〃 | 〃 |
| * Duck | 4•¼—17 〃 | Richet | 1899 |
| * *Uria troile* | 6—12 〃 | Bohr | 1897 |
| * White rat | 2 〃 6 sec | Bert | 1870 |
| * Water rat (*Arvicola amphibium*) | 2 〃 17 〃 | 〃 | 〃 |
| * Rabbit | 3 〃 | 〃 | 〃 |
| * Cat | 3 〃 | 〃 | 〃 |
| * Dog | 4 〃 25 〃 | 〃 | 〃 |
| Sea elephant (*Mirounga angustirostris*) | 6 〃 48 〃 | Harnisch | 1937 |
| * Muskrat (*Ondatra zibethica*) | 12 〃 | Irving | 1939 |
| * Beaver (*Castor canadensis*) | 15 〃 | Irving and Orr | 1935 |
| * Harbor seal (*Phoca vitulina*) | 15 〃 | Millais | 1906 |
| * Gray seal (*Halichoerus grypus*) | 15 〃 | 〃 | 〃 |
| Florida Manatee (*Trichechus latirostris*) | 16 〃 20 〃 | Parker | 1922 |
| New Zealand humbacked whale (*Megaptera nodosa*) | not over ½ hours | Lillie | 1910 |
| Fin whale (*Balaenoptera plupalus*) | 20 min | Ommanney | 1932 |
| Blue whale (*Balaenoptera musculus*) | 50 〃 | Andrews | 1916 |
| Finback whale (*Balaenoptera physalus*) | 30 〃 | Allen | 1916 |
| Sperm whale (*Physeter catodon*) | 1—1•½ hours | Millais | 1906 |
| Bottle-nosed whale (*Hyperoodon rostratus*) | 2 hours | Gray | 1822 |
| Man, average swimmer | 1 min | Irving | 1932 |
| Man, pearl and sponge divers | up to 2 min | Irving | 1932 |

* average time to last movement by forced submersion.

the course of chase are often defective in exactness and sometimes exaggerated. The duration of diving differs naturally in different species of whales. Within the same species, it is said that the larger the whale, the longer the duration.

LAURIE[1] observed that blue whales dive on being pursued, for 10 minutes or more and that the harpooned blue whales stay submerged as long as 25 minutes. He gathered from conversations with Norwegian gunners that the average time of dives of the blue whales which are not alarmed is about 10 minutes, and when being chased or harpooned, the submergence may last as long as half an hour. According to OMMANNEY[2], although the usual duration of dives of the blue and fin whales is between 10 and 20 minutes, there are instances to be known of longer dives than this. The Greenland right whale is said to be able to make dives lasting up to 45 minutes[3].

SCHOLANDER[4] observed how a swimming fin whale unfrightened and at liberty respires and dives, by means of a kymograph on board, on which the sounds of "spoutings" were recorded. Diagrams taken from two different fin whales showed that the duration of each dive was within the range of 4 to 6 minutes. However, he added that he himself had seen the longer dives of fin whales, lasting for 10 to

12 minutes. This author further cited the following particulars which were furnished by the experienced gunners at BRÖDRENE SÄBJÖRNSEN's whaling station at Steinshamn (Norway):

Fin whale (*Balaenoptera physalus*) 5–6 min. (10–12–15 min.)
Blue whale (*Balaenoptera musculus*) 7–8 min.
Piked whale (*Balaenoptera acutorostrata*) 6–7 min.
Bottle-nosed whale (*Hyperoodon rostratus*) 30–45 min.
Sperm whale (*Physeter catodon*) 30–45 min., 20–40 spoutings during the course of 7–8 minutes.

It is evident that the sperm whale and perhaps particularly the bottle-nosed whale are the best prolonged divers. There would be no doubt that the sperm whale is able to dive spontaneously for at least one hour and the bottle-nosed whale perhaps has still greater diving ability than the sperm whale. In contrast, the balaenopterids like the blue and fin whale show in general, far poorer diving ability. Their diving ability is not so surprising even when compared with that of other aquatic animals like the seal.

Depth—. OMMANNEY's opinion[2] which denies the deep dives of whales deeper than 130 feet (40 metres) on the ground of the risk of caison sickness, seems to have no reason, for the whales may have the sufficient reason for not being afraid of the caison disease. He said, "The limiting factor in any dive is the danger of caison disease upon returning suddenly from the depths where the excess hydrostatic pressure is greater than 1.25 atmospheres (2.25 atmosphers absolute pressure)..... it seems improbable that any organism could withstand the effect of swift release from pressure greater than 5 atmospheres—corresponding to a depth of 132 feet." He continued further," It may be said then, that a whale probably does not descend to depths much greater than 130 feet, but can remain below for periods of up to half an hour." (pp. 357–8). Later, we will consider whether the danger of caison sickness is actually present when whales make a swift floating from the tremendously great depths.

LAURIE[1] assumed that a blue or fin whale does not go further down deeper than 100 metres, as the krill, food of these whales is not found in sufficiently large quantities below this depth. However he mentioned that he was informed of a dead sperm whale which was found off the Peruvian coast entangled in a submarine cable at a depth of 900 metres. Probably it was caught in the cable while it was in activity, and finally drowned.

As SCHOLANDER[4] pointed out, the depth estimated by length and the direction of run-out harpoon line, is uncertain and happens to contain a great error. He measured actually the depths up to which harpooned fin whales submerged, by means of a specially deviced manometer attached to the harpoon line, 75 metres behind the harpoon; to this length of 75 metres he assumed the depth of 25 metres. By this method, he measured the depths on five fin whales and the manometer readings were corresponding to 85, 105, 135, 230 and 355 metres respectively, every with the exception of 25 metres to be added.

A sperm and a bottle-nosed whale are known to live for a great part on the cephalopods belonging to deep sea species. Therefore it may be very likely that these whales habitually go down to depths deeper than in the occasional deep dives of the fin whale. Although direct evidence is still lacking, HEESEN's report[5] on the troubles caused to submarine cables by whales seems to provide some indirect evidences. He inquired into the troubles caused by whales to the submarine cables belonging to the Western Union Telegraph Co. and four other agencies. According to this report, the troubles so far recorded had amounted to 14 cases, of which the majority were caused by the sperm whales at great depths but a few were caused by balaenopterids in comparatively shallow waters. Six out of the 14 cases took place at the depth of about 900 metres with the sperm whale as the culprit and the deepest record was 1130 metres.

The cables were hardly injured in almost all the cases, and the high correlation was held between the freshness of the carcass and the time-lapse from the time when the interruption of telegraph was found to the time when the cable was hauled up to the sea surface for repair and the carcass of the culprit discovered concurrently. It is very probable from these facts that whales were entangled in the cable when they were alive, perhaps while being engaged in pursuit of cephalopods. It seems almost certain that the sperm whale, and perhaps the bottle-nosed whale go habitually down much deeper than so far imagined.

## THE OXYGEN STORED AND CONSUMED

It will be unnecessary to say that a whale is in a state of complete apnoea during submergence. A whale may expel the air but cannot inhale it, and the expelling the air means nothing but a loss of oxygen for a submerged whale. It is inconceivable that a whale should be capable of taking oxygen from the surrounding sea water through any part of its body surface. Therefore it is completely evident that the oxygen to be consumed during a dive must be stored within the whale's body, till the commencement of the dive. We shall now consider how much oxygen can be stored in the whale's body and how much will be consumed in a single dive, in other words, we are going to inquire into the balance-sheet of oxygen within the whale's body during a submergence.

OMMANNEY[2] expressed his opinion that if whale's metabolism is in any way comparable with that of man, a whale will need to take down, when diving for half an hour, a store of oxygen at least twenty times greater than its lungs can contain. Certainly, the balance of oxygen storage and its consumption in whales seems at first sight, to be not coincident. However the oxygen store cannot be restricted to the lungs. Thus, such local consideration will serve little to elucidate this problem. Moreover, we must consider some special mechanism which would maintain the whale's metabolism in a condition of deficient oxygen. At any rate, we will consider in this chapter, the purely arithmetical relation between the oxygen store and its consumption.

## Oxygen Stores

The lung air, blood and tissues should be considered to serve as the important oxygen depots. In addition to the absolute amount of oxygen in these depots, it should be important to know how completely these depots can be utilized.

Lung oxygen—. The direct measurement of the vital capacity* or the lung capacity of whales is impracticable. However, LAURIE[1] found that the lungs of a blue whale weighing 122 tons and 27.2 meters long, are in proportion to the body weight, lighter by about the half than those of man; the lungs of this whale weighed 1226 kg, or around 1% of its body weight, whereas human lungs average over 2 %. Assuming that the lung capacity is directly proportional to the lung weight in whales as in man, and taking the vital capacity as 3.5 litres in man of 70 kg body weight, he estimated the vital capacity of the whale under consideration at 3050 litres. This blue whale, by the way, the particulars of which were kindly supplied to the Discovery Committee by Capt. SÖRLLE in 1926, will be cited hereafter as the "SÖRLLE's blue whale" or merely as the "SÖRLLE's whale".

On the other hand, the measurements of lung capacity (maximum inflation capacity) of dead whales made by SCHOLANDER[4] by means of a specially big dry gasmeter gave the following results for three different fin whales:

Fin whale 50 feet, ca. 30 tons, lung capacity 800 litres (2.7 1/kg%)
——— 68 〃 〃 60 〃 ——— 1500 〃 (2.5 〃 )
——— 72 〃 〃 70 〃 ——— 2000 〃 (2.8 〃 )

From these figures, we can see that the LAURIE's assumption of the vital capacity of the SÖRLLE's whale is not too conservative; this is corresponding to just 2.5 1/kg% of the body weight. It would be closer to the lung capacity rather than the vital capacity.

Meanwhile, what should be thought about the ventilation efficiency of the whale lungs? Human tidal air (ventilating amount of air at each usual breath) is only 300 to 400 cc, or only 6 to 8% of the lung capacity of 5.3 litres. LAURIE[1] pointed out that the microscopic feature of the section of whale lung is characterized by the greater thickness of the walls of infundibula and alveoli as compared to those of man or pig. Such circumstances of lung tissues may effect more complete renewal of the alveoli air at each breath. As a further reason to suppose that a whale makes a more complete exchange of the lung air than any other animals, he referred to the forcible spoutings (blasts of expiration). According to his observations[1], a spouting of a whale in the Antarctic, is clearly visible as a column of condensed vapor of a height of 20 feet lasting for half a second and the noise is audible on a still day for a distance of at least half a mile.

SCHOLANDER[4] suggested from the results of the respiratory experiment, a high

* Human vital capacity, i.e., the greatest amount of air which can be taken in, after the most forcible expiration, averages 3.5 litres in man of 70 kg body weight, that is, corresponding to 5 1/kg% of the body weight. The lung capacity, which is the vital capacity added by the residual air in the lungs, is about 3/2 times as great as the vital capacity, that is, it measures 5.3 litres in man of the same size—corresponding to 7.5 1/kg% of the body weight.

ventilation efficiency of a small porpoise (*Phocaena communis*) weighing 19 kg. The tidal air of this porpoise was surprisingly constant, amounting in every respiration to 1 litre. From a certain experimental ground, he put the lung capacity of this *Phocaena* at 1400 cc at a high estimate. From this results, it seems certain that the porpoise makes a very efficient renewal of the lung air and we might well presume that this is also the case in greater whales, though the actual evidence is not available. It seems not to be strange to assume the great ability of renewal of the lung air for such animals like whales which must hold breath for a long time from their habit of life and during this time, must depend only upon the limited store of oxygen, instead of enjoying the continuous ventilation of the lung air as do land animals.

It is a general observation that the whale dives after inspiration. There are two types of respiratory pause in general; the inspiratory and the expiratory one. HARNISCH[6] observed in sea lions and sea elephants, that the respiratory pause is generally inspiratory but occasionally expiratory. He frequently observed that the animals expelled air at the beginning of a dive. According to SCHOLANDER[4], it was a constant occurrence in his respiratory experiments that a seal (*Halichoerus grypus*) expires strongly and dives with very little air in the lungs. However, he noticed no such phenomenon in the porpoise (*Phocaena communis*). He noted that *Phocaena* and the fin whale dive on an inspiration, as probably do all other whales.

As for the depletion of oxygen in the lungs, BOHR[7] found in *Uria troile* 1.5 to 4% oxygen in the lung air in suffocation. RICHET[8] found that the lung air of ducks contains 1.3 to 2.9% oxygen at the premortal suffocation. With whales however, no actual determination of this kind is available.

It may be well to regard the partial pressure of oxygen in the whale lungs as 150 mm Hg at the outset of submergence. This is being decreased in the course of apnoea activity. However, in considering the decrease in the oxygen partial pressure in the lungs, it may be necessary to take into account that whales make a deep dive. If a whale stays submerged at a depth of 90 meters, the hydrostatic pressure exerting upon the whale body, will be 10 atmospheric pressures, and assuming that every part of the whale body undergoes this pressure uniformly, the air in the lungs must be compressed by the gas law, to 1/10 the volume that it has occupied at the sea surface. This compression of the lung air may result in the more complete use of oxygen. LAURIE[1] said that a partial pressure of 60 mm Hg is sufficient to keep the blood of most animals above 80% saturation with oxygen, and that, if the whale stays at the depth of 90 meters where the oxygen partial prssure in the whale lungs is assumed to have been decreased to 60 mm Hg, then, when the whale returns to the surface, the oxygen in this whale lungs would be at a partial pressure of only 6 mm Hg.

If the matter is as discussed by LAURIE, the residual oxygen is to be only 0.8% of the lung air, or 96% of the total amount of oxygen formerly inspired is to be utilized. However, this discussion seems to be speculative. Because, even if the oxygen partial pressure of 60 mm, at the total pressure of 760 mm Hg were suffici-

ent to keep the blood to 80% saturation with oxygen, the same oxygen partial pressure cannot be sure to effect the same degree of oxygen saturation of the blood when the total pressure is 10 times as great.

Blood oxygen—. The blood volume ranges from 7 to 10% of the body weight in most mammals and birds, and is somewhat greater in diving animals. According to IRVING[9], the blood volume is about 10% in the duck and muskrat, i.e., a little greater than those found for land mammals. In two seals, the same author found about 10% blood volume. SCHOLANDER[4] also found that the blood volume is 9 to 10% in the seals. As for the whales, there is found little information. However, in the SÖRLLE's blue whale, it was estimated at 8000 litres*—corresponding to 6.5 % of the body weight. This value is smaller than that of man, 7–8%. However we may presume that the blood volume of whales would be at least, as much as that of man and more probably around 10%, in considering that the bleeding of such a gigantic animal as a whale is not easy to be carried out completely.

The oxygen capacity of the blood in diving animals is rather well known. Table 2 has mainly been based upon compilations by IRVING[10] and SCHOLANDER[4]. In most mammals and birds, the oxygen capacity is between 15 and 20 v/v% of the blood, and in general, the diving animals have greater oxygen capacity than land animals.

Table 2. Oxygen Capacity of Blood in Equilibrium with Air

| Animal | Oxygen capacity % | Authority | |
|---|---|---|---|
| Pigeon | 21.2 | Wastl and Leiner | 1931 |
| Hen | 11.2 | Winterstein | 1921 |
| Duck | 16.9 | Wastl and Leiner | 1931 |
| *Uria troile* | 26.0 | Bohr | 1897 |
| Dog | 21.8 | Irving | 1939 |
| Beaver | 17.7 | 〃 | 〃 |
| Muskrat | 25.0 | 〃 | 〃 |
| Seal (*Phoca vitulina*) | 29.3 | 〃 | 〃 |
| Sealion (*Eumetopias stelleri*) | 19.8 | Florkin and Redfield | 1931 |
| *Phocaena communis* | 20.5 | Green and Redfield | 1933 |
| *Tursiops truncatus* | 31.8 | 〃 〃 | 〃 |
| Blue and fin whale | 14.1 | Laurie | 1933 |
| Sperm whale | 29.1 | Green and Redfield | 1933 |
| Man | 20.7 | Irving | 1939 |

A few informations are available as to the oxygen capacity of the whale blood. However, most of them are concerned with carcass' blood drawn up to many hours after death. As SCHOLANDER pointed out, the whale usually bleeds in the sea from the shot wounds for many hours and therefore there remains a suspicion that the

* This was supplied by Capt. Sörlle to the Discovery Committee in 1926, together with the other particulars of this whale.

sea water may enter the blood during towing, through the harpoon wounds and belly cuts. In addition, there is another uncertainness to be not able to judge what changes may have occurred in the blood as the result of bleeding and sedimentation. However in whales, the oppotunity is almost rare to do with a alive individual.

SCHOLANDER[4] found the oxygen capacity of the blood in a living *Phocaena communis* weighing 19 kg, to be 15.6%. LAURIE[1] obtained the result that the oxygen capacity averages 14% in the blood from 7 fresh blue and fin whales. SCHOLANDER[4] obtained the values of between 12 and 28% oxygen capacity in quite fresh materials from greater whales. He noted also that the carcass blood of some sperm whale and that of some *Phocaena* showed both 24% oxygen capacity. He mentioned that a reasonable figure for the oxygen capacity of the fin whale blood would be some 20 or 25% and that the sperm whale and the bottle-nosed whale bloods might reasonably be assumed to have a higher oxygen capacity, about 30% or more than in seal. This might be probable, but few evidences have been so far presented.

Tissue oxygen—. We will under this item, chiefly inspect the oxygen depots in muscle pigments and in tissue fluids. As will be seen later, the fat is capable to retain much oxygen, but it is excluded here. The fat as an oxygen depot, will be discussed later in another chapter.

(1). Muscle pigments. In most mammals and birds, myoglobin and other respiratory pigments in the muscle amount to about 15% of the blood hemoglobin. The myoglobin as a store of oxygen, is characterized by the fact that it has a lower oxygen tension of saturation than the hemoglobin and that its oxygen capacity is much smaller as compared with hemoglobin. TEORELL[11] demonstrated in a solution of the crystalline myoglobin from horse the myoglobin at pH 7.4 is already half-saturated at the oxygen partial pressure of 3.26 mm Hg, whereas the hemoglobin is not half-saturated until 20 mm. VERZÁR[12] assumed a maximum oxygen tension in the resting muscle, of 19 mm, which would give 90% oxygen saturation of the myoglobin. MILLIKAN[13], by comparing the oxygen capacity of the myoglobin in a normal mammalian heart and other red muscle, and its oxygen consumption, deduced that the oxygen store of myoglobin could not last the tissue more than 10 or 15 seconds at rest and not more than 2 or 3 seconds at high activity. He also showed in cat that the myoglobin acts as a short time oxygen store to tide the muscle over from one contraction to the next.

Thus, although it should be certain that the myoglobin serves as an oxygen store, it seems that we cannot expect much from it. However, muscle pigments are known to be more plenty in the whale's muscle, especially in the muscle of the sperm and the bottle-nosed whale. TAWARA[14] determined the amount of the myoglobin and cytochromes in the muscle of sperm and sei whale, and found that the amount of myoglobin is twice as much in the muscle of sei whale, and 8 to 9 times as much in the sperm whale, as in beef. But no remarkable difference was found to exist in the content of the cytochromes between the muscles of the named three and that of the blue whale. From these result, we may well presume that myoglobin serves as more important oxygen store in the sperm whale and perhaps in the bottle-nosed

whale. Nevertheless, the oxygen stored in these depots cannot be expected to last the muscle more than 1 minute at the moderate activity.

(2). Tissue fluid oxygen. Excepting the blood, the tissue fluid of the whale may be estimated at 60% of the body Weight. As the fat content is considerably high in the whale body, this may be rather a high estimate. On the other hand, the solubility of oxygen in water is 2.44 cc (reduced to standard state) per 100 cc of water, at 35°C and at the oxygen partial pressure of 760 mm Hg. Therefore, when the water is brought into contact with the atmospheric air in which the oxygen partial pressure is about 150 mm Hg, the water will dissolve about 0.5 v/v% oxygen at 35°C. Accordingly, the tissue fluid of the whale body may be also able to dissolve some 0.5 v/v% oxygen when the whale is at the surface. IRVING[10] and SCHOLANDER[4] adopted this value of 0.5%.

At first sight, this estimation seems to be the very truth, if disregarding some discrepancy due to the fact that the tissue fluid is a solution in which various substances are already dissolved. However, it will prove that it is not the case, when we take into account that the tissue fluid does not come into contact with the atmospheric air but comes into contact with the blood. The blood can retain much oxygen owing to its chemical combining power of oxygen; its oxygen capacity is usually over 15%, when it is brought in equilibrium with 150 mm oxygen partial pressure of the atmospheric air. In other words, we can regard the blood as a concentrated aqueous solution of oxygen, though it is heterogeneous. Thus, when the tissue fluid is brought into contact with blood whose oxygen concentration is over 15 %, it is evident that the tissue fluid will dissolve oxygen more than 0.5% of its volume. If the solubility of oxygen in the tissue fluid is sufficiently great and the blood is so constructed that the equilibrium is established when the oxygen concentration of the blood comes to equal to that of the tissue fluid, then the tissue fluid can finally dissolve oxygen up to 15 v/v%. However, this will be far from the truth. It will be only right to say that the amount of oxygen which should dissolve in the tissue fluid is determined by the actual oxygen partial pressure of the blood.

## Energy Requirement during Submergence

There are found a few informations related to this problem about whales. Indeed, how much energy is consumed during submergence of a whale, it would be hard to estimate. However, we are to enumerate at least, as the dissipation sources of energy, the energy consumption for basal metabolism, that for the translational movement of the whale body and that for the vertical motion against the buoyant or gravitational forces. Beside these kinds of energy dissipation, the balaenopterids will consume their energy in collecting a large amount of the krill, and the sperm and bottle-nosed whale, for struggling with the great cephalopods. The harpooned whale, which immediately dives deeply with full speed, pulling the harpoon line, must use a considerably large amount of energy. The net dissipation of energy of these kinds is very hard or almost impracticable to computate. Thus for the moment, we will consider only the first named three, that is, the energy requirement

for basal metabolism, that for translation and that for the vertical motion.

Energy for basal metabolism—. LAURIE[1] assumed a very little energy for the basal metabolism (b. m.) of the whale. Assuming that the energy requirement for b. m. of a warm-blooded animal is a function of the body surface alone, and that it is 1000 Calories per day per square metre of the body surface, and taking the body surface of the SÖRLLE's blue whale as 275 $m^2$, he calculated the energy requirement for b. m. of this whale at 275000 Cal/day. If we reduce this value to per body weight, it amounts only to 0.0016 Cal/kg, min—corresponding to about 0.3 cc/kg, min of oxygen consumed, as the oxygen consumption is 200 cc per Calorie produced. While the oxygen requirement for b. m. is 4.3 cc/kg, min, in man of 70 kg body weight.

The present author wonders, however, whether LUBNER's surface law which LAURIE applied to the estimation of energy for b. m., is so universal as to be applicable to any warm-blooded animal regardless of the species and the living environment. Surely we may feel to go too far with this law, when LAURIE applied it to such an aquatic homoiotherms as a whale. The medium in which the whale lives is much different in physical properties from that for land animals. The heat capacity of water is some 3200 times as great as that for the same volume of air, at ordinary temperatures and pressures. It would be partly for this reason that warm-blooded animals are not suitable to live in water. On the other hand, we must certainly remember that the whale is enveloped in the thick blubber which undoubtedly constitutes a good insulating wall. If it is the case that, owing to the insulating capability of the blubber, the energy consumption for b. m. is kept at the same level, for a whale in its water life and for a land animal in its land life, LAURIE's estimate will be right, although it seems doubtful to the author. Nevertheless, the author will tentatively adopt the value given by LAURIE, for our later calculations.

Work for submergence—. A whale must at first, sink its body against buoyancy of the water. Now we will consider the quasi-static work which must be done by the whale (or the potential energy aquired by the whale), when it submerges vertically against the buoyancy. If we write $w$ and $v$ for the weight and volume of a whale and $\rho$ and $\rho'$ for the specific gravity (sp. G.) of the whale body and the sea water respectively, the buoyancy which acts upon the whale body, is

$$B = v\rho' = w\frac{\rho'}{\rho} \quad \cdots\cdots (1)$$

Accordingly, the whale body undergoes an upward force $B-w$. So that, quasi-static work which must be done against this force for a vertical submergence through the length $l$, is

$$A = w\left(\frac{\rho'}{\rho} - 1\right)l, \quad \cdots\cdots (2)$$

where $A$ denotes the work. In Equation 2, if $\rho' > \rho$, the work is positive and if inverse, the work is negative, that is, work is necessary for floating, not for sinking.

In our calculations, it may be sufficient to assume that the sp. G. of the sea water

is 1.025. As for the sp. G. of the whale body, there is found no description but only one. In this old description, W. SCORESBY[15] mentioned that the Greenland right whale is very nearly of the same sp. G. as sea water, some individuals sinking and others barely floating when dead. In the modern whaling as we know, it is the usual practice that the whale after death, is inflated with the compressed air in order to prevent from sinking, but this procedure occasionally can be omitted in the sperm whale.

Therefore we may well regard the sp. G. of the whale body when it is dead, as a little greater or little smaller than that of the sea water. However, we must pay attention to the fact that this statement is concerned with the sp. G. of the whale *when dead*. When the whale is dead, the respiratory muscle being relaxed, it would not be strange to suppose that only a part of the air remains in the lungs of the carcass, the other part having escaped through the respiratory ducts.

We will now proceed to the numerical consideration of this problem, taking the example of the SÖRLLE's blue whale weighing 122000 kg, whose lung capacity being 3050 litres according to LAURIE. If we assume that, when this whale is dead, 2000 litres of air escaped from its lungs and therefore, 1050 litres remained, and that, in such circumstances, the sp. G. of this whale is 1.028 (a little greater than the sp. G. of sea water), then the sp. G. of this whale must have been 1.011, when with a lungful air of 3050 litres, ignoring the weight of the air. Whereas, the air in the lungs must decrease in volume, in accordance with the depths up to which the whale dives. If the decrease in volume results according to the gas law, we can easily find the depth where the sp. G. of this whale becomes identical with that of the sea water. Thus, it may be advisable to find at first, the depth at which the sp. G. of the both comes to be equal. Because, work is needed only to this depth and beyond this point deeper, no work is necessary to be done by the whale; the vertical sinking must be a spontaneous process. As a result of calculation, we find this point, at the depth of 12 meters from the surface, in the afore-mentioned conditions.

On the other hand, starting from the assumption that the sp. G. of the carcass is 1.022 (a little smaller than that of sea water) and the others are all the same as before, we obtain the equilibrium point to be 34 meters in depth.

These calculations were carried out on the assumption that the solid and the liquid parts of the body undergo no compression and the hydrostatic pressures increase at the rate of 1 atmospheric pressure for every 10 meters of water depth.

The above-mentioned calculations suggest that a whale need not make so much effort to dive. In divings, whales have parhaps a native skill to render the buoyancy minimized, by exposing a part of their body out of the water; by doing so, they can make the work of submergence minimized or negative. In fact, they habitually bow their back and then dive. The depth of 12 or 34 metres will be a trifle for a whale as long as 27 metres. It seems therefore that the work of submergence need not be considered as an important source of energy dissipation.

Work for emergence—. As has been mentioned in the preceding section, the

buoyant force comes to in equilibrium with the gravitational force, at the depth of about 34 metres, on the second assumption that the sp. G. of the whale is 1.022 when it is dead. So that, at the depths below, the gravitational must overcome the buoyant force, in other words, the work must be done for floating not for sinking. Let us now consider the work that the SÖRLLE's whale must do for floating. It is a matter of course that Equation 2 is valid for this calculation, but the opposite form is more convenient for use, that is,

$$A = w(1 - \frac{\rho'}{\rho})l, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where $A$ denotes the work for floating, $w$, the weight of the whale, and $\rho$ and $\rho'$, are the sp. G. of the whale body and the sea water respectively.

Assumptions and other particulars to be used in the calculation are as follows:

(1) that the whale weighing 122000 kg begins to float from the depth of 1000 metres; this assumption may be true for the sperm whale;

(2) that the sp. G. of the carcass with 1050 litres lung air is 1.022; hence, the sp. G. of the same whale with no lung air amounts to 1.031;

(3) that this value of 1.031 is taken for the sp. G. of the solid-liquid portion of the whale body as a whole and this value of the sp. G. does not change with changing hydrostatic pressures; that is, the sp. G. of the whale body excluding the lung air is regarded as a constant throughout our floating process; this assumption may be true, because of the very small compressibility of the solid and liquid;

(4) that the total mass of the lung gases remains unchanged throughout the process and it takes the volume of 3000 litres at the temperature of the whale body and at 1 atm.; this assumption is made with the intention that nitrogen gas is hardly affected by metabolism and the loss of oxygen will be compensated by the production of carbon dioxide.

To simplify the calculations, the work of floating is divided into two parts; namely, the work of lifting the solid-liquid portion of the whale body ($A_1$) and the work of lifting the gaseous portion ($A_2$), that is,

$$A = A_1 + A_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$A_1$ is obtained by the simple numerical calculation of Equation 3, where $w = 122000$ kg (neglecting the weight of the lung air), $\rho = 1.031$, $\rho' = 1.025$ and $l = 1000$ m, and we find the work, $A_1 = 707600$ kg-m.

Next, let us calculate, $A_2$, the work for lifting the gas in the lungs. If we write $w'$, for the weight of the gas in the lungs, $\rho_0$ and $\rho'$ for the sp. G. of the gas and the sea water respectively and $dl$ for the distance through which the gas is lifted, the infinitesimal work for lifting the gas, is

$$dA_2 = w'(1 - \frac{\rho'}{\rho_0})dl \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

In Equation 5, since the sp. G. of the gas, $\rho_0$ is a function of the water depth, $l$, we

must give the relation between $\rho_0$ and $l$. At the first, we write as our definition,

$$\rho_0 = \frac{w'}{v'}, \quad \cdots\cdots (6)$$

where $v'$ denotes the volume of the gas in the lungs. Furthermore we obtain from the preceding assumption (4), ignoring the effect of the temperature change,

$$pv' = 3000 \text{ (1, atm)}, \cdots\cdots (7)$$

where $p$ is the hydrostatic pressure which must be equal to the lung air tension. Finally the relation between the hydrostatic pressures and the water depths can be approximately stated as

$$p = \frac{l}{10} + 1. \cdots\cdots (8)$$

For combining Equation 5, 6, 7 and 8, we obtain

$$dA_2 = w'\left[1 - \frac{30000\rho'}{w'(l+10)}\right]dl. \cdots\cdots (9)$$

Integlation of Equation 9 gives

$$A_2 = w'l\Big]_0^{1000} - 30000\rho' \ln(l+10)\Big]_0^{1000}, \cdots\cdots (10)$$

and by the the numerical calculation of Equation 10, taking $w' = 3.6$ kg (as the sp. G. of the lung air is 0.0012), we find $A_2 = -209120$ kg-m, for the work of lifting the air in the lungs. Thus, we have the total amount of the work for floating the whale

$$A = A_1 + A_2 = 498480 \text{ kg-m}$$

This is the quasi-static work which must be done by the SÖRLLE's whale, for floating through 1000 metres; around 500000 kg-m—corresponding to 1167 Calories (as 1 kg-m=1/427 Cal).

Meanwhile, we can hardly find the way to estimate the efficiency, or the conversion factor of the muscle engine of the whale. SCHOLANDER[4] estimated it at 20 %. However, the present author was told that the efficiency of the muscle in ordinary sportsmen averages 22% and in well-trained experts, it reachs about 30% for their familiar exercise.* A whale must be an expert in divings. Therefore, we may be less erroneous to regard the efficiency of the muscle of whales as 30%, than to regard it as 20%.

Assuming it to be 30%, 3890 Calories is necessary for the work under consideration—corresponding to 778 litres of oxygen consumption (0.2 1/Cal). This amount of oxygen is greater than the oxygen stored in the lungs, namely, 610 litres

* From the conversation of Dr. Prof. H. HAGIWARA (Sports Physiology), Department of Sportings, Faculty of Education, Hiroshima University.

with respect to the SÖRLLE's whale. In summary, we can see from the above calculations that the amount of oxygen consumed for floating may be not a trifle for the sperm whale, which must preserve this amount for the last travel of emergence.

Towing resistance —. HANSSEN calculated the towing resistance of the SÖRLLE's whale (cit. SCHOLANDER) and obtained the results that the towing resistance is equivalent to 1.5 horse powers (Hp.) at a speed of 3 knots, 6.5 Hps. at 5 knots and 46.8 Hps. at 10 knots.

As for the swimming speed of whales, it may be said that the blue and fin whales can swim at the sea surface with repeated shallow dives, at the speed of 15 to 20 knots, when they are on a long voyage or being chased. However, it is difficult to tell at what speed they swim under the water. We have no evidence as to whether whales swim at less speeds under the water than at the surface. However, they display only a part of their body out of the water, even when swimming at the surface. Hence, it seems reasonably to be presumed that they could swim under the water at nearly the same speed as at the surface.

On the other hand, in view of the energy economy, it is improbable that the whales swim under the water at an unnecessarily high speed and thus they consume energy for useless work. Then, it appears most probable that they swim under the water, at the speed of 5 to 10 knots, taking account of the collecting a large mass of the krill (for the balaenopterids) and of the pursuing the great cephalopods (for the sperm and bottle-nosed whale). On the basis of this reasoning, we estimate conservatively that the average power for swimming of a submerged whale is 10 Hps. If assumed so, 10 Hps. is 45000 kg-m/min, or 105 Cal/min, as 1 Hp. = 75 kg-m/sec and 1 kg-m = 1/427 Cal. Taking the efficiency of the muscle engine as 30% as before, the energy consumption for swimming under the water averages 350 Cal/min, or the oxygen required for this work comes to 70 l/min, in average.

## The Balance of Oxygen

In the preceding section, we have attempted to give the total amount of oxygen which a whale must carry with them in diving, and have found that several factors which would constitute the basis of our quantitative considerations remain still unknown. Indeed, there is still much to be learnt until we can strictly speak of the maximum oxygen content of the whale body and further, how completely this amount could be used up during submergence.

For the present, we can merely estimate from discussed above as follows, taking the example of the SÖRLLE's whale:

(1). The lung oxygen amounts to 610 litres, because the total lung air is 3050 litres, according to the LAURIE's estimate. Then, the utilizable oxygen at high estimate, can be 550 litres, taking the depletion as 90%.

(2). The maximum estimate of the blood oxygen amounts to 2440 litres, taking the blood volume as 10% of the body weight, and the oxygen capacity as 20% of the blood. As for the sperm or bottle-nosed whale, it would amount to 3660 litres, if they are of the same size as the SÖRLLE's whale (as the oxygen capacity is as-

sumed to be 30%). However, these estimated values may be considerably far from the truth, for we have not taken into account the degree of arterialisation of the blood. Therefore, when considering various depressing factors, the blood oxygen available during diving might be far less; the present author will estimate it at 70 % of the amount estimated above.

(3). The oxygen store ascribable to the muscle pigments may be negligible, as has been mentioned in the corresponding section.

(4). The tissue fluid oxygen is hardly to be estimated as has been already discussed. However, the present author is strongly inclined to believe that the tissue flluid is reasonably considered to dissolve oxygen up to the solubility at the oxygen partial pressure of 760 mm Hg, when it is brought into contact with blood. On the other hand, it may be also improbable that the whole amount of the fluids becomes saturated with oxygen within a comparatively short time. At the same time, we must take into account that the tissue fluid is a solution in which various substances are already dissolved. Thus under favorable conditions, if the tissue fluid has dissolved 1% oxygen at the onset of diving, then the total amount of the tissue fluid oxygen amounts to 730 litres, as the tissue fluid is assumed to be 60% of the body weight. Taking the depletion as 90%, the utilizable oxygen is estimated at 660 litres.

Thus, the total amount of oxygen which would be available for the metabolism during submergence of a whale sums up as follows:

| | For SÖRLLE's whale | For sperm whale of the same size |
|---|---|---|
| Lung oxygen | 550 litres | 550 litres |
| Blood oxygen | 1700 | 2560 |
| Tissue fluid oxygen | 660 | 660 |
| Sum | 2910 litres | 3770 litres |

We must now recall that a whale must reserve the oxygen necessary for the floating. This will be at least, 780 litres, if the whale emerges from the depth of 1000 metres, as calculated in the preceding section, and about 160 litres, if it emerges from 200 metres. Subtracting these amounts from the total available oxygen, the remainder will be 2750 litres for the SÖRLLE's whale (assuming from 200 m), and 3000 litres for the sperm whale of the same size as the SÖRLLE's whale.

On the other hand, the oxygen consumption rate during submergence will sum up to about 110 litres per minute; 38 litres for basal metabolism and 70 litres for swimming. At this consumption rate, the SÖRLLE's whale could last for 25 minutes dive with this depot, and the sperm whale endures 27 minutes dive. However, we must remember that this oxygen consumption rate is the minimum to be calculated. With this rate, whales cannot even change their swimming direction. The work for towing resistance contains no other energy dissipation than the translational motion at constant velocity. Concerning the energy for basal metabolism, the said figure is probably fairly underestimated, as has been noted in the preceding section.

Nevertheless, it seems interesting to us that the calculated and the experienced

diving durations are of the same order, for the balaenopterids. Thus it may be possible to consider that a dive lasting for several or ten and several minutes may be not severe for the balaenopterids, if viewed merely from the point of oxygen economy, disregarding the sensitivity to the excess of carbon dioxide. On the contrary, for the sperm and bottle-nosed whale, it is evident that their long diving duration cannot be explained from the estimated oxygen depot alone.

## THE MECHANISMS WHICH WOULD REDUCE THE OXYGEN CONSUMPTION DURING DIVING

Diving bradycardia—. Since P. BERT[16] first discovered it in ducks in 1870, the bradycardia (heart beat slowing) during submergence has been recorded by many authors in a variety of mammals and birds, and even in essencially non-diving animals including man. SCHOLANDER[4] observed the pronounced and reflective bradycardia in the submergence of seals; the decrease in heat frequency from 150 to 10 per minute. However, the same author could not detect the bradycardia in the artificial apnoea experiment of a porpoise (*Phocaena communis*). He said, "Palpatory and auscultatory there was some respiratory arrhythmia of the heart during apnoea, but no apnoea bradycardia could be induced, nor was this possible in a previous real submersion experiment on another porpoise. This is in sharp contrast to the immediate occurrence of bradycardia in seals during such experiments."

There is yet no proof as to whether bradycardia is induced in the greater whales, but it may probably be not, if inferred from the SCHOLANDER's observation on *Phocaena*. The direct consequence of the bradycardia will be, of course the retardation of the blood flow, and therefore it may lead to the general shortage of oxygen in every tissue and organ.

In connection with this problem, IRVING and his school suported the hypothesis of "the differential distribution of oxygen during diving". He said, "The storage of oxygen is inadequate to provide for its indiscriminate use by all of the tissues. No shift of metabolism can compensate for the lack of oxygen, but differential control of oxygen might reasonably serve to maintain the brain, allowing less sensitive tissues or those with fair capacity for anaerobic metabolism to do without oxygen." OMMANNEY[2] also called attention to the fact that, provided the brain and nervous system are kept supplied with oxygen, a man may incur a dept of oxygen in his tissues equal to ten times the amount of oxygen in his lnugs.

Some experiments speaking for this hypothesis are also known. However, the observations which apparently offer the decided evidence for this hypothesis were made by IRVING himself. The measurements were carried out in anaesthetized beavers, on the blood circulation both in brain and in muscles by insufflation apnoea and apnoea by clamping the trachea. He found in this case, that the apnoea bradycardia is accompanied with the reduced blood circulation in the muscle but increased blood circulation in the brain. They ascribed this effect to the peripheral vasoconstriction.

Retia mirabilia—. The retia mirabilia are the most remarkable anatomical feature in whales. These vascular networks, of which HUNTER[18] mentioned for the first time, have been noticed by many anatomists and have caused much speculation. However, in this connection, we may be referred to the study of OMMANNEY[2]. He gave the detailed description of the thoracic, basicranial and other retia, in the anatomical investigation of two fin whales' foetuses.

The thoracic rete is the vast and long-extended mass of networks of the vascular capillaries, located along the vertibral column from the 1st cervical to the 6th dorsal vertebra. These networks moreover, pass between the transverse processes into the neural canal where they are in connection with the intraspinal retia. The basicranial rete lies at the base of the skull.

The fundamental features of these retia are given by OMMANNEY[2]. The rete is the dense mass of networks of vascular capillaries which are in open communication everywhere with certain arteries and veins, embedded in its capillary system. This capillary system of the rete is supplied with blood by its own arteries from the main arterial system and drained through its own veins into the main venous system. The rete does not impede the blood flow on the course of main blood vessels, but intervenes between the main arteries and veins. In this respect, it gives an impression of the arterio-venous shunt, but it is not merely the constrictor mechanism. The rete is as a whole, embedded in a large quantity of fat and enveloped in stout connective tissues. Thus it has no relation with any surrounding organ or tissues, and therefore it appears to be an independent organ.

The retia mirabilia or structures similar to these, are found in all mammals which are capable of diving and of staying submerged for long periods, that is, in seals, porpoises, dolphins and whales. The *sirenia* which do not have the habit of remaining submerged so long, do not possess these vascular networks.

OMMANNEY[2] is of the opinion that these vascular networks are most likely to be concerned in some way with gaseous exchange. He said, "In this connection, the situation of the networks near the respiratory centre and on the course of main blood vessels and also around the brain and nerve chord is extremely suggestive. It has already been mentioned that the masses of the rete are abundantly charged with fat. Oxygen is very soluble in fats. It may be imagined that some chemical mechanism exists for a more speedy transfer of oxygen from the blood to the fatty masses of the retia, which may thus act as an oxygen storage mechanism. The retia may in fact be capable of forming a sort of accessory lung. Since the vascular networks are under the control of the somatic nervous system, they may possibly be engorged with blood at will from the posterior thoracic artery, and this blood may perhaps be discharged at will again into the posterior thoracic vein."

## THE POSSIBLE MEANING OF EXISTENCE OF FAT IN WHALES

As for the solubility of oxygen in fat, OMMANNEY[2] made reference, in connection with the probable functions of retia, to the book of LEWKOWITSCH[19], in which the solubility is given as follows:

After 3 days' exposure at normal temperatures and pressures.

100 parts of cod liver oil absorb 6.382 $O_2$
" whale oil " 8.266
" sperm oil " 1.629
" Japan fish oil " 8.194

From these figures, we can see that the whale oil is of the highest solubility of oxygen, while the fish oil is also capable of dissolving almost the same amount of oxygen. Remarkable is the fact that the sperm oil can absorb far less oxygen when compared with the other samples. In this connection, OMMANNEY[2] expressed his opinion as follows: if the sperm whale makes a longer dive than the fin whale, as is usually said by whalers and accepted by some writers (he did not believe in this tendency of the sperm whale, at that time), one should expect to find in the sperm oil a high oxygen solubility; the results tabulated above however, show that the solubility is decidedly lower in the sperm oil than in other oils; the retia of the sperm whale were not yet examined in detail, but a superficial inspection indicates that they are of even greater extent than in the fin whale; this might be possible, if the oxygen solubility of the sperm oil is less than that of the fin whale.

ISHIKAWA[20)21)22)] measured not only the solubility but also the absorption rate of oxygen and other respiratory gases in a variety of oily substances and water at 35°C and at various pressures. Thus, he ascertained that (1) the solubility of oxygen does not differ so much among the sperm waxes, whale oil and usual glycerides at each given pressure, but among all the samples employed, sperm waxes show the highest oxygen solubility; (2) for all the solvents investigated, HENRY-RAOULT's law on the dilute solution of the gas is valid; (3) in every material used, the absorption rate of a gas can be expressed by the formula, $y=A(1-e^{-\alpha t})$, where $y$ is the amount

Table 3. Solubility of gases in oily substances and water

| Material | Solubility (cc/100g, atm)* | | |
|---|---|---|---|
| | $CO_2$ | $O_2$ | $N_2$ |
| Sperm whale head oil | 130.0 | 14.4 | 9.12 |
| Sperm whale body oil | 122.0 | 14.6 | 8.82 |
| Fin whale oil | 118.0 | — | — |
| Blue whale oil | — | 13.0 | 7.41 |
| Olive oil | 124.0 | 13.3 | 8.21 |
| Camellia oil | 121.0 | 12.7 | 6.92 |
| Castor oil | 106.0 | 8.8 | 5.59 |
| Oleic acid | 127.0 | — | — |
| Oleyl alcohol | 103.0 | — | — |
| Liquid paraffin | 85.0 | 13.6 | 7.27 |
| Dist. water | 61.0 | 2.4 | 1.24 |
| 1% saline water | 60.0 | — | — |

* The solubility is expressed in terms of cc (reduced to standard state) of a gas per 100 g of a material and per 1 atmospheric pressure of the gas.

Table 4. Absorption rate of gases in oily substances and in water

| Material | Initial absorption rate (cc/100 g, atm, min)* | | |
|---|---|---|---|
| | $CO_2$ | $O_2$ | $N_2$ |
| Sperm whale head oil | 32.5 | 1.40 | 1.40 |
| Sperm whale body oil | 27.0 | 1.19 | 1.01 |
| Fin whale oil | 18.2 | — | — |
| Blue whale oil | — | 0.49 | 0.60 |
| Olive oil | 8.4 | 0.55 | 0.46 |
| Camellia oil | 6.7 | 0.46 | 0.24 |
| Liquid paraffin | 4.9 | 0.26 | 0.15 |
| Castor oil | 3.0 | 0.088 | 0.043 |
| Dist. water | 36.8 | 0.20 | 0.19 |
| 1% saline water | 32.6 | — | — |
| Oleic acid | 14.1 | — | — |
| Oleyl alcohol | 10.7 | — | — |

* The absorption rate is expressed in terms of cc (reduced to standard state) of a gas per 100 g of a material, per 1 atmospheric pressure of the gas, and per minute.

of gas absorbed up to the time $t$, $A$, the saturated amount at given temperature and pressure, $\alpha$, a constant disregarding the pressures applied but characteristic of the kinds of solvent and (4) absorption rate thus calculated from the said formula is very different with different kinds of material used (see Table 3 and 4).

The solubility of oxygen in the sperm wax is 14.5 cc (reduced to standard state) per 100 g of solvent and per 1 atm. of the oxygen partial pressures, in contrast to the solubility in water of 2.4 cc, that is, about 6 times as great in the sperm wax as in water. While, the solubility of oxygen in the whale oil is 13.0 cc by the same unit. As for the absorption rate, the initial absorption rate is 7 times as great in the sperm head wax as in water, and some 2.5 times as great in the blue whale oil as in water.

Here must be noted that the result obtained by ISHIKAWA is inconsistent with that of LEWKOWITSCH, with respect to the oxygen solubility of sperm wax. However, we must call to mind that the former is obtained at 35°C (near the body temperature of the whale) and the latter is at the "normal temperatures." The sperm wax as we know, is the crystalline soft solid at room temperatures and on the contrary, is the liquid of high fluidity; the viscosity of the sperm wax at 35°C, measured by ISHIKAWA[20] is lower than all the oily materials employed. Then, it is possible that the sperm wax at the normal temperatures, is of the low solubility of oxygen, or more probably, of so slow rate to absorb oxygen, that the saturation cannot be reached by far, by the 3 days' exposure to the atmosphere. To all intents and purposes however, it may be sufficient to say that the wax should act at the body temperatures of the whale, not at the normal temperatures.

It will be clearly seen from the aforementioned, that the solubility of oxygen in oils particularly in the whale wax, is much higher than that in water, and that the absorption rate is especially greater for the wax than for the whale oils and other

glycerides. It might then, the next problem, whether oxygen can be really dissolved in oils or wax in the living whales. No direct information is available about this problem. However, the present author would refer to the fact which was found by the post-mortem examinations of patients of the caison sickness. LAURIE[1], discussing the possibility of getting this disease in whales, made reference to the book of HILL[23], who gave the detailed description of this disease. According to HILL, the nitrogen bubbles are found in various parts of the corpses, but most frequently in the fat. The fat in all cases of decompression is honey-combed like whisked egg white.

Then, it may be unreasonable to consider that oxygen does not dissolve in fat, in spite of the foregoing fact that nitrogen is actually dissolved in fat of living individuals. It may be probably that the oxygen partial pressure is far predominant over that of nitrogen within the whale body, when the whale respires on the surface. Because the physical solubility of oxygen is about twice as great as that of nitrogen, at the same partial pressure and temperature, in both aqueous tissue fluids and fats. Moreover, the blood may be regarded as a concentrated aqueous solution of oxygen, on account of its chemical combining power for oxygen; it is usually 15–20% oxygen solution in equilibrium with air. On the contrary, nitrogen can be dissolved in the blood, as far as only 1% at the same atmospheric air. In a word, the blood can carry 15–20% oxygen and some 1% nitrogen. If the blood should transfer the oxygen and nitrogen to the fats in the whale body, this transfer would occur according to their own solubilities and their partial pressures. Thus it may be possible to expect that the ratio of oxygen to nitrogen which are given up from the blood to the fat, is 15–20: 0.5.

The solubility of oxygen in fat as seen from Table 3, is 14.5 v/w% in the sperm wax, and 13.0% in the blue whale oil, at the oxygen partial pressure of 760 mm. Hg and at 35°. So that, at the partial pressure of 150 mm Hg (under the atmospheric air), the solubility comes to about 3%. However, as has been already discussed in the foregoing chapter (see p. 359), the dissolution of oxygen in fat does not occur in equilibrium with the oxygen partial pressure of the air, but would occur in equilibrium with the oxygen concentration of the blood. Thus, it appears reasonable to the author that the fat can dissolve oxygen as far as the solubility at the oxygen partial pressure of 760 mm Hg. If that is the case, this value of 14.5 or 13 % is that which is comparable to the oxygen capacity of the usual blood.

While, the fat content of the whale is in general, 25 to 30% of the body weight. Hence, for a moment, if the fat content is taken as 20% and this amount of fat could dissolve *in vivo*, oxygen as far as 10%, we will have 2440 litres of oxygen for the SÖRLLE's whale weighing 122000 kg. That is just the same amount as the blood might store at high estimate. This arithmetic answer may be of course, far from the truth. Because, the fatty system is in general, not so abundantly supplied with blood vessels that the system can take much oxygen from the blood during a short period. However we can not avoid the conclusion that the fat would dissolve more or less, oxygen from the blood or from the lymph. Especially it is hardly supposed

that the retia and any system like these, which contain much fat and at the same time, are supplied with rich circulation, do not store any oxygen from the blood.

In reference to the problem in question, the result obtained by SCHOLANDER[4] in the artificial apnoea experiment of a porpoise (*Phocaena communis*), is very suggestive. He summarized as follows: the oxygen intake of this little *Phocaena* weighing 19 kg, was exceedingly high, between 400 and 500 cc per minute, this amount is more than for a man of 70 kg body weight, or twice the consumption per kg body weight of a seal; during whole of the experiment, the whale lay immoval and the high oxygen "consumption" remained unchanged for 33 minutes. The present author cannot but inquire whether this great amount of oxygen (13–15 litres for 33 min) was really consumed by the 33 minutes metabolism at rest, of this 19 kg porpoise. Certainly, this reminds me of some phenomena like the latent heat.

At the end of this chapter, it may be of interest to see the peculiar habit of respiration of the whale. As the whale comes up to the surface, it expires forcibly with half opened nostrils (by the way, the sperm whale has only one nostril), and then immediately inspires with nostrils wide opened. According to LAURIE[1], the total time from the commencement of the expiratory blast (spouting) to the closing of the blow holes, averages 1.5 sec; 0.6 sec for expiration and 0.9 sec for inspiration. Now after this inspiration, follows an immediate dive. But this dive is not long nor deep. In general, between two succesive deep dives (sounding) intervene 5–6 times shallow dives in the balaenopterids, and 20–40 times, occasionally 80 times shallow dives in the sperm whale. It is the point to which the author will refer, why the whale inevitably takes a respiratory pause after every inspirations, by means of the shallow dive. This shallow dive seems to be of no effect but rendering the inspiration effective. Indeed by this procedure, the oxygen inspired in the lungs must be made more soluble in the tissue fluid and in the fat, as a result of the increased hydrostatic pressures.

## DEEP DIVING AND CAISON DISEASE

One of the problems which arises from the deep diving of the whales, is how the whales avoid getting the caison sickness, when they emerge from the deep submersion. During diving, the nitrogen gas goes into solution in the blood and distributes itself in the liquid parts of the body by the diffusion. The solubility of nitrogen in the aqueous fluid or fat is proportional to the hydrostatic pressures which the whales undergo, and the hydrostatic pressures are increased at the rate of 1 atmospheric pressure for every 10 metres of water depths. Then the amount of nitrogen soluble in the body fluid of the whale is to be determined by the depth and the time of submergence. If the whale should rise too quickly to the surface, there would not be time enough to emit for the nitrogen out of the blood or fluid, then it would form bubbles in the blood vessels and other tissues; it is the cause of the gas embolism. By the way, the oxygen and carbon dioxide dissolved in the body fluids cannot in ordinary cases, give rise to the caison disease. Because the former is soon consumed and the latter is of greater solubility. Thus the nitrogen and other innert

gases (but these latters being the minor importance) remain to be the object of the considerations.

Human divers can descend to a depth where the hydrostatic pressure is 2.25 atm. (all our expressions being by the absolute pressure), i.e., to the depth of 12.5 metres, and return to the surface with no care, but at the greater depths than this, precautions for prevention of the gas embolism must be taken depending on the duration of the exposure to the increased pressures.

Many authors have been hitherto engaged in searching this problem. However it seems to have proved that this question is a kind of the bugbear. OMMANNEY[2] denied the deep divings of the whale to avoid this difficulty (see p. 353), and LAURIE[1] answered this question by finding the "X-organism", which is according to him, living in the blood of the whale and eliminates the nitrogen. It may be however improbable that any microbe inhabits in the blood of healthy animals. Moreover, the results of the same author indicate that this small creature expends a considerable amount of oxygen, together with the nitrogen. So that the existence of these organisms in the whale blood becomes more and more doubtful, partly because of the said reasonings and partly because of the more decided fact that these organisms are again found by no other investigator.

In considering the possibility of getting caison sickness in whales, it may be advantageous to divide the diving into two categories, in order to avoid confusion; that is, diving with a lungful air (mono-breath dive) and diving with the respiratory apparatus (poly-breath dive). In the poly-breath dive, the source of nitrogen is practically unlimited and the nitrogen is continued to be supplied without limitation. On the contrary, in the mono-breath dive, it is limited and the amount of nitrogen is evidently that which is already dissolved in the body at the normal atmosphere, plus that in the lung air. Thus it is evident that, in the poly-breath dive, the amount of nitrogen soluble in the body fluids is a function of the depth and duration of submergence, but in the mono-breath dive, the maximum amount of nitrogen soluble in the body fluids is only dependent upon the limited amount aforementioned. Thus, the question whether the whale can get caison sickness, is to be answered by the fact whether the amount of nitrogen in a lungful air of this whale is sufficient to give rise to this disease.

Let us now, proceed to the numerical consideration of this problem, taking the SÖRLLE's whale as an example. At a starting point, let the aqueous fluid of this whale be 70% including the blood, and fat be 25% (the oil produced from this whale was 22.75% by SÖRLLE) of the body weight. The solubility of nitrogen in water and in the whale oil is from Table 3, 1% and 6% respectively in round numbers, under the atmosphere. Then the maximum amount of nitrogen soluble in the whale body sums up to 2680 litres. On the other hand, the nitrogen in the lungs (the lung capacity being 3050 litres by LAURIE) is 2440 litres. This amount of 2440 litres is that which will be already soluble in the whale body before this whale reaches the depth of 10 metres.

Thus when the whale rises quickly from the great depths, it cannot undergo

more effect than that which would be exerted upon the whale on emerging from the depth of 10 metres, with respect to the gas embolism. The present author at least, cannot believe that the mono-breath dive might be accompanied by the caison disease.

## SUMMARY

In this article, two problems are mainly argued namely, (i) what mechanisms enable the whale to endure submergence so much longer than other animals, and (ii) how it can avoid getting caison disease when it makes the swift rising from the great depths.

(1). The duration and depth of submergence in whales are surveyed by making reference to the reliable informations.

(2). The balance of the oxygen store and its consumption is considered. As oxygen depots, the lung air, the blood and tissue fluids are enumerated, and as the oxygen consumption sources, basal metabolism, translational movement and vertical motion are taken into account. Thus, it is almost certain that the storage of oxygen cannot cancel the oxygen consumption during a long dive of the sperm whale or the bottle-nosed whale.

(3). The mechanisms which would reduce the oxygen consumption during apnoea are considered. The retia mirabilia are the most remarkable structures which may serve as the control mechanisms of oxygen distribution, preventing the brain and nervous system from the oxygen deficiency.

(4). The author discussed the problem as to whether the fat in the whale body is possible to act as the oxygen storage or to be concerned with gaseous exchange.

(5). It was argued whether the whale can get caison disease, when it makes a swift rising from the great depths. The present author was strongly inclined to conclude that any animal cannot get caison disease in its "mono-breath dive."

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# Cytological and Histological Studies on Abnormal Follicles of Mature Mice

Tsunenori NAKAMURA

*Laboratory of Reproduction Physiology, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Plates 1–13; Text-figs. 1–4; Tables 1–13)

## CONTENTS

(I) INTRODUCTION ..... 375
(II) MATERIAL AND METHODS ..... 378
(III) RESULTS OF OBSERVATIONS ..... 379
PART I. ON ABNORMAL FOLLICLES IN MATURE OVARIES OF MICE AT DIFFERENT PHASES OF OESTROUS CYCLE ..... 379
(1) Normal Mature Follicles ..... 379
(2) Abnormal Mature Follicles ..... 381
(3) Polyovular Follicles ..... 383
(4) Polyovular Follicles in Immature Mice ..... 385
PART II. EFFECTS OF SEX STIMULATING HORMONES ON ABNORMAL FOLLICLES OF MICE ..... 386
(1) Effects of the Mixture of Anterior Pituitary Sex Gland Stimulating Hormones and the Sex Gland Stimulating Hormones from Placenta ..... 387
(2) Effects of the Mixture of Anterior Pituitary Sex Gland Stimulating Hormones and Sex Gland Stimulating Hormones from Placenta and Pregnant Mare-Serum of Horses ..... 389
PART III. NUMBER OF ABERRANT FOLLICLES IN RELATION TO THE STRAIN OF MICE ..... 396
PART IV. SOME HISTOCHEMICAL OBSERVATIONS ON NORMAL AND ABNORMAL OVA IN MOUSE OVARIES ..... 401
(1) Glycogen-Contents in Ova ..... 402
(2) RNA-Contents in Ova ..... 403
(IV) DISCUSSION ..... 403
(V) SUMMARY ..... 407
(VI) LITERATURE CITED ..... 408
(VII) LEGENDS FOR PLATES 1 TO 13 ..... 410

## (I) INTRODUCTION

During these thirty years, the remarkable growth of knowledge of the physiology of mammalian ova in several parts of the world has serried to a great extent the analysis of very complicated mechanisms of ovulation following advances of cytological methods, on the basis of fundamental physiological and morphological studies of ovulation.

The cyclic changes of the sex organ are more pronounced and complicated in

mammals than in other animals; they are known as an oestrous cycle, and occur at different intervals of days or weeks according to the species. It is only during a period of the oestrus that the mature ova are produced in the ovary, and the female becomes sexually receptive, so that fertilization can occur. The oestrous cycle has so far been most closely studied in rodents, particularly in the Muridae, since they breed in every season through the year in caged conditions. Mature female mouse ovulates repeatedly about once every about 5 days from the age of 60 days. The typical oestrous cycle begins with the active secretion of the gonadotrophic hormone from the anterior pituitary. This secretion commences when the animal has grown to maturity. It is thought that at this early stage it is the follicle stimulating hormones which is produced in great quantity. The follicles stimulating hormones of the anterior pituitary induce rapid growth of Graafian follicles, and a part of this growth is achieved by mitosis and another part by the secretion into the antrum of the follicular fluid and of oestrogen. At a final point, the rate of oestrogen secretion has a depressing effect on the rate of pituitary secretion and increases the rate of secretion of interstitial cell stimulating hormone. Thus, about the time of ovulation the supply of follicle stimulating hormone and of oestrogen is diminishing, and the interstitial cell stimulating hormone is then mainly responsible for the luteinization of cells of the ruptured Graafian follicles to form the corpus luteum. KNAUER (1896) seems to be the first who studied the mechanism of ovulation, and STOCARD & PAPANICOLAOU (1917) dealt with the maturation process in the Graafian follicles using the smear method with vaginal fluid in the guinea pig. ALLEN (1923) and HISAW (1947) put foreward a theory on the oestrous cycle in the Muridae. Also, MAKINO (1941) reported the cytological features of normal follicles in mature ovaries of mice (*Mus musculus* L.). On the other hand, ASAMI (1920), HARTMAN (1926), ENGLE (1927), TAKEWAKI (1937), LANE (1938), PLISKE (1940), MAKINO & SIGEMORO (1946) and SIGEMORO (1947) reported on atretic follicles which contained abnormal ova with polyovular follicles in mice and rats; they showed that a great number of ova were produced in mammalian ovaries during the oestrous, but the majority of them underwent degeneration, only a few attaining maturation, and developing into functional ova. To be regret, however, most of these investigators have dealt with merely morphological observations of the atretic ova, and failed to inquiry into their behavior and changes involving the maturation phases leading to their disintegration. Further detailed observations have been left for the analysis of mechanism by which atretic ova would be produced. Here, further investigation is invited to study effects of some hormonal substances upon the formation and fate of atretic ova.

The present author's former study has been dealt with cytological observations on abnormal mature ova which followed the maturation course in the normal sexual cycle of mature mice. The results indicated that the degeneration of abnormal ova was caused by the disintegration taking place in the egg cell, in accordance to the view emphasied by ENGLE (1927). On the other hand, there are another veiw that the disintegration occurs in graulosa cells reported by ASAMI (1920), BRANCA (1925),

EREUD & VEDDER (1938) and PLISKE (1949). This view is of suggestive that the disintegration may be due to the influence of sex stimulating hormones. Probably, a follicle stimulating hormone from the anterior pituitary would induce rapid growth of the Graafian follicle; the growth may be partly achieved by multiplication of follicle cells and partly by the hormonal secretion into the follicular fluid. It has been known that the follicular fluid contains a large amount of oestrogen; the latter may increase the mitotic activity of follicle cells. Since the pituitary induces the active secretion of the follicular fluid as well as oestrogen, it may be the oestrogen that practically causes the real growth of the follicle. Based on the results obtained the suggestion may be possible that the ovum which undergoes degeneration without being growing into the functional follicle would be produced under the influence of certain sex hormones. But, a question remains as to whether any kind of hormones affects, or controls, the growth of Oögonium, or primary and secondary follicles.

Further, the present author has studied cytologically with particular concern the influences of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormone from the placenta upon atretic mature ova in mice. Also, the influence of the pregnant mare serum of horses on the abnormal mature ova of mice has been investigated by the author in order to see the fate of abnormal ova.

Furthermore, the investgation has been extended by the author in order to learn the difference in number of normal, abnormal mature ova and polyovular follicles in mature ovaries of mice derived from different strains obtainable in different laboratories of Japan, since no such investigation has been carried out by any author.

Some histochemical studies of normal and abnormal mature ova in mouse ovaries have been a further subject of investigation undertaken by the author. There have been published recently several reports on histochemical studies of atretic ova in the mammalian ovary by HARTER (1948), VINCENT & DORNFELD (1948), DEANE (1952), ISHIDA (1953, 1954), and some others. The present study has been carried out in some detailed for the analysis of histochemical nature in normal and abnormal ova, use being made of two different mouse strains.

The data obtained in the present investigations was described being separated into four parts in this paper. Part I is devoted to the cytological studies on normal and abnormal mature ova, and polyovular follicles in mature ovaries of *Mus musculus* (Swiss albinos): they were observed at different phases of the oestrous cycle with the use of the vaginal smear method.

In Part II is described an cytological effect of sex stimulating hormones on large abnormal follicles and ova in *Mus musculus* (Swiss albinos). The study was subdivided into two parts. The first dealt with the effect of the mixture of anterior pituitary sex gland stimulating hormone and sex gland stimulating hormones from placenta upon the fate of abnormal follicles. The second was the effect of the mixed hormones as above and the pregnant mare serum of horses upon abnormal follicles. It was hoped in these studies to examine whether or not the administration of sex stimulating hormones derived from the anterior pituitary gland, placenta gland

and pregnant mare serum of horses would reduce the number of abnormal mature ova and polyovular follicles.

Part III describes the results of the cytological investigation of some comparative features of normal, abnormal mature ova and polyovular follicles in mature ovaries in the following four mouse strains; Swiss albinos, D-240 albinos, B-72 albinos and dd/Ma albinos.

Part IV gives accounts of the histochemical observations of normal and abnormal mature ovaries of mice studied at four stages of the oestrous cycle. The occurrence and localization of glycogen and RNA were the subjects mainly dealt with in ovaries of *Mus musculus* (Swiss albinos and D-240 albinos).

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## (II) MATERIAL AND METHODS

Unmated mature female mice (*Mus musculus*) at 70 to 120 days of age furnished the material for the present study; they are Swiss-albinos derived from the Agriculture Department of Tokyo University and D-240 albinos, B-72 albinos and dd/Ma albinos derived from the Makino Laboratory, Zoological Institute, Hokkaido University. They were all purely bred in the author's laboratory.

At the start of experiments, vaginal smears were daily made from each mouse and inspected under the microscope. Individuals which showed a normal sexual cycle were used as material for study. The sexual cycle of mice generally shows a considerable variation. Especially, a few mice from S-strain and D-240 strain were observed to shown an abnormal sexual cycle. They showed an abnormally long dioestrus cycle. They were not used as material for the present study. In every specimen, weight was recorded every 5 days during the course of experiments.

The methods for study are summarized as follows:

Part I. The ovaries taken from 30 individuals of S-strain mice were used as material. They were removed and fixed with Bouin's solution. The sections were made according to the usual paraffin method, and stained with Heidenhain's iron-haematoxylin.

Part II. Twenty-five individuals of S-strain mice were received injections of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormones from placenta ("Synaphorin", Teikoku Hormone MFG., Co.) 2 R.U. every day for 3 successive days in a period of the oestrus stage. Starting on the 5 days before the oestrus stage, 25 individuals were injected with "Synaphorin" 2R.U. per day for 3 successive days and again with pregnant mare serum of horses, ("Serotropin", Teikoku Hormone MFG., Co.) 50 I.U. Twenty-four hours after injection, the ovaries were removed and fixed with Bouin's solution. The sections were made

according to the usual paraffin methods and stained with Heidenhain's iron-haematoxylin. Seventeen individuals were reserved as controls.

Part III. A comparative cytological investigation was made with ovaries derived from 30 individuals of Swiss albinos, from 30 individuals of D-240 strain mice, from 20 individuals of dd/Ma strain mice, and from 20 individuals of B-72 strain mice. They were fixed with Bouin's solution, and treated with the method as described above.

Part IV. For histochemical investigations, the ovaries taken from 8 individuals of Swiss albinos and 2 individuals of D-240 strain mice provided the material. Fixing fluids for the demonstration of glycogen and RNA were 95 % alcohol, Zenker's fluid and 10 % formalin. The sections were made according to the usual paraffin method, and stained with Schiff's periodic acid modified by Lilie for the observation of glycogen. For RNA, thionin staining method was adopted.

The work was carried out during a period ranging from early spring to late autum every year, since the animals breed rather well in those seasons.

## (III) RESULTS OF OBSERVATIONS

Part 1. On abnormal follicles in mature ovaries of mice at different phases of the oestrous cycle

(1) Normal mature follicles of mice

The first maturation division of the oocyte is completed prior to ovulation in the mouse ovary as in other mammals. The normal maturation division of the oocyte was observed only in the Graafian follicle which follows advancing regularly the course of the maturation. Functional mature ovum showing a normal sexual cycle was found in the Graafian follicle before its rupture. Such a mature follicle was observed occupying a position at the periphery of the ovary. The ovum lying in the Graafian follicle is surrounded by a gelatine membrane, called zona pellucida, and the latter again by corona radiata. Surrounding the corona radiata there are granulosa cells which enclose a huge cavity filled up with the fluid, liquor folliculi. In the mature follicle being 217 to 290$\mu$ in diameter, the mature ovum has a diameter of 51 to 82$\mu$ under a fixed condition. MAKINO (1941) reported that the egg of mouse (*Mus musculus* L.) has a diameter ranging from 65 to 70$\mu$. SOBOTTA and BURCKHARD (1910) reported that the diameter of rat egg are 60 to 65$\mu$. YAMANE (1937) are measured 110 to 120$\mu$ in diameter of the rabbit egg. The size of the mouse egg nearly approximate that of the rat. The observations indicated that the egg diameter seems to differ by different strains of mice under study: dd/Ma mice has a diameter ranging 75 to 80$\mu$, the follicle being 286 to 350$\mu$, the B-72 mice 78 to 80$\mu$, the follicle being 286 to 340$\mu$, D-240 mice 73 to 80$\mu$, the follicle being 243 to 357$\mu$. It is evident from the above results that there is no striking difference in egg-diameter by difference of mouse strain. Following the penetration of the spermatozoon the ovum generally reduces in diameter.

After the disappearance of the nuclear membrane, the chromosome arrange

on the equatorial plate to form the first spindle in the ovum. After the division of chromosomes, the outer pole of the spindle which upheaves on the egg surface gets extruded as the first polar body. The ovum thus undergoes the first maturation division prior to ovulation and still further advances in its maturation course up to the metaphase stage of the second division. The maturation process in the ovum stops at this stage, untill insemination occurs following ovulation. The detailed descriptions involving the maturation phenomena in the normal mature ovum in mice were given in the paper of MAKINO (1941). It has been generally accepted that ovulation occurs prior to the completion of the second maturation division, and that during ovulation the process leading to the second division is arrested, lying quiescent, as it were in the metaphase condition. Insemination is therefore a necessary anticipation to the second maturation division. It is therefore evident that the results of this study is of considerable significance in giving a cytological proof to the results experimentally attained by YAMANE (1930, 1935a, b, 1937) in the rabbit egg *in vitro*. He reported that spermatozoa showed a twofold action consisting in dispersing sticky follicular cell-mass surrounding the ovum and activating the second maturation division in artificial insemination *in vitro* with rabbit ova. The formation of the second polar body following the penetration of spermatozoon into the ovum was deduced to be a proteolytic action of enzyme presumably of the trypsin group. In an ovary, the author observed an ovum which shows the first and second polar bodies in the unmated mouse. This may be an example showing abnormal maturation divisions in an atretic follicle (Fig. 42).

The author has chanced to observe some Graafian follicles of a normal appearance which show a great antrum.

The changes of the nucleus of the ovum advancing towards the first maturation division seems to proceed successively during a short duration. After the disappearance of the nuclear membrane has successively taken place, the chromsomes make their arrangement on the equatorial plate and thus the first polar spindle is formed (Figs. 1-14). After the separation of chromsomes the outer pole of the spindle which upheaves on the egg surface, gets extruded as the first polar body (Figs. 15-16). The ovum enters into the second maturation division carrying the first polar body, and lies practically free from the granulosa cells as well from the corona radiata cells, being suspended in a naked state in the liquor folliculi (Figs. 17-18). It advances in its maturation course up to the metaphase stage of the second division. The maturation course in the ovum stops at this stage, until insemination occurs following ovulation.

For the sake of convenience, the stages of the normal follicle are divided into three general types as follows:

(1) Primary follicles; any follicle having one or more cell layers with no antrum or a few antrum.

(2) Secondary follicles; any follicle with a prominent antrum and the ovum starting in the maturation division.

(3) Corpora lutea; a crude categorgy including any follicular structure after rup-

ture.

Primary follicles refer to small primary follicles with six to eight rows of granulosa cells, but in which the antrum folliculi has not yet been fully formed. This type are abundant in ovaries of young mice (Fig. 67).

Secondary follicles show all follicles which have an antrum in each (Fig. 11). In the present study, the secondary follicles advancing towards the maturation course were observed in 157 examples from 30 individuals under study. They showed diameter of 200 to 350$\mu$. One hundred-nine follicles from 28 ovaries of 14 individuals were at the phase of proestrus; 20 follicles from 10 ovaries of 5 individuals were at the stage of oestrus; 28 follicles from 14 ovaries of 7 individuals showed the dioestrus stage (Table 1).

The number of normal mature ova in average at four stages of the oestrous cycles was 7.8 at the proestrus, 4.0 at the oestrus, 4.0 at the metoestrus and 0 at the dioestrus. The ovulation seems to be taken place during a period from about 12 to 20 hours in the oestrous stage. The proestrus stage is just before the rupture of ova. The number of normal mature ova at this stage is 7.8 on an average with a variation range from 1 to 9 (Table 10). SATO (1959) dealing with some observations on natural ovulation in mature ova, reported the average number of mature ova as 8.2 in D-240 strain *in vitro*.

(2) Abnormal mature follicles

The atretic follicles are generally divided into two types. Type 1 refers to the small primary follicle with several rows of granulosa cells: some of them shows the antrum folliculi consisting of several rows of granulosa cells, while some others contain no antrum folliculi. Type 2 deals with all follicles which advance to maturation phase; they mostly have no antrum, though a few show an antrum. The follicles belonging to type 1 are found in the ovum before the maturation course, whereas type 2 in the ovum undergoing the maturation division. The object of this work is to study the morphology of the ovary of type 2.

The number of ovary of type 2 as observed in 60 ovaries was 85, while normal mature follicles were 157 in number, as given in Table 1. In the phase of the oestrous cycle, fifty-eight follicles from 28 ovaries of 14 individuals were at the proestrus stage; 15 follicles from 10 ovaries were the oestrus stage; 8 follicles from 14 ovaries were the metoestrus stage; 4 follicles from 8 ovaries were the dioestrus stage.

The number in average of abnormal ova in four stages of the oestrous cycle were 4.1 at the proestrus, 3.0 at the oestrus, 1.1 at the metoestrus, and 1.0 at the dioestrus. Total number of abnormal ova was 2.8 in an average, while normal mature ova were 5.2 (Table 10). It was observed based on the vaginal smear method that the atretic follicles at the proestrus stage are very frequent through the oestrous cycle. The data presented here suggest a numerical relation between the abnormal and normal mature ova in an ovary.

Some of the abnormal mature ova showed the chromosomes which arrange on the equatorial plate with the first polar spindle, having no nuclear membrane and

antrum (Figs. 19-36). Some others showed the second polar spindle at metaphase accompanied by the first polar body in the follicle with a few antra (Figs. 37-41): this type of abnormality is rather common in mature ovaries. Some others showed the completion of the second division offering the second polar body, though the antrum folliculi has not been formed (Fig. 42). There were also found abnormalties of chromosomes (Figs. 43-44), and the first division showing a tetra-polar spindle (Figs. 60-61). The irregularity in shape of the ovum was also common; in the extreme case, the ovum contained many vacuoles in the ooplasm (Figs. 58-59).

ENGLE (1927) in the mouse and SIGEMORO (1947) in the rat have emphasized that in the course of degeneration the disintegration takes place in the egg body. On the other hand, ASAMI (1920), BRANKA (1925), EREUD & VEDDER (1938) and PLISKE (1940), all in rats, have pointed out that the disintegration occurs in the granulosa cells. Based on the data so far obtained, it seems probable that in the course of degeneration the disintegration takes place in both in the egg body and in the granulosa cells and as a result it exerts some influence on the hormone secretion in the mature ovary. Especially, the hormone preparations admininstered probably affect only the follicles of larger size as shown type 2.

From the results of observation with ovaries of 30 specimens of mice, it is evident that those showing abnormality in egg body were found in 73 examples, while those showing abnormality in granulosa cells were observed in 12 examples in total. In the eggs at the proestrus stage from 14 individuals, the ratio of the egg body abnormality to granulosa cell abnormality was 49 : 9, in those at the oestrus stage, from 5 individual the ratio was 11 : 4, in those at the metoestrus stage from 7 individuals the ratio was 7 : 1, and in the eggs from the dioestrus stage from 4 individuals the ratio was 4 : 0 (Table 2).

A suggestion is made that the disintegration of granulosa cells and egg body is due to the influence of the sex stimulating hormone. This may be proved by the investigation on the effects of the sex stimulating hormones on the abnormal mature ova on the sexual cycle of mice, the details being described in the latter section.

The atretic follicles in the mammalian ovary have been studied from the morphological view point by many investigators (KINGERY 1914, LOEB 1911, ASAMI 1920, ALLEN 1923, BRANCA 1925, ENGLE 1927, ALLEN, KOUNTZ & FRANCIES 1925, EREUD & VEDDER 1938, PLISKE 1940, SIGEMORO 1947, PINCUS & ENZMANN 1947, HISAW 1948, ISHIDA 1953, 54). ALLEN, KOUNTZ & FRANCIES (1925) reported the cyclic destruction of ovarian ova in the oestrous cycle. ASAMI (1920) ENGLE (1927), are a quantitative study of follicular atresia in the mouse, Engle (1927) stated that all of the atretic follicles are divided into two general types. Type I refers to small primary follicle, with from two to six or eight rows of cells in the granulosa, but in which the antrum follicles has not yet formed. Type II refers to all follicles which have an antrum, these usually ranging between 250$\mu$ and 750$\mu$, although those with one layer are occasionally found. The results of observation on the atretic follicles at four stages of the oestrous cycle showed that there was a cyclic variation, both in number of pseudomaturation spindles and in the total number

of atretic follicles. The destruction is at its highest peak during the first day of the dioestrus and in its lowest on the second day. The curves showing the number of atretic follicles do not indicate the same type of variation at four stages of early pregnancy. This seems to suggest a rather low ovarian activity: it is comparable to the condition at pseudopregnancy. PINCUS & ENZMANN (1947) studied atresia of ovarian eggs in the rabbit. They reported that four definite types of atretic follices were found and the percentage of atresia among the young oocyte is low (10%). In the follicles of larger size about 60 percent was atretic ovary and the hormone administration affected only the largest follicles and their ova. SIGEMORO (1947) studied abnormal follicles in rats, and reported that the widely encountered features of atresia were observed in the sign occuring in the egg nucleus. Recently, ISHIDA (1953, 54) made some morphological observations of atretic ova in adult rats and some other domestic animals and reported that the primary follicles seemed to undergo destruction, showing their ova which were shrinking. The first sign of degeneration of ova in the secondary and Graafian follicles was the appearance of a pseudomaturation spindle.

(3) Polyovular follicles of mice

Extensive literature regarding polyovular follicles was presented in the reviews by HARTMAN (1926), ENGLE (1927), DEDERER (1934), TAKEWAKI (1937), LANE (1938), MAKINO & SIGEMORO (1946) and SIGEMORO (1947) in the mature and immature mammals. HARTMAN (1926) seems to be the first who studied the mechanism of polyovular follicles in the majority of mammals, including the maruspials and man. He expressed the view in connection with the polyovular follicle and twining. ENGLE (1927) reported 18 polyovular follicles based on one hundred ovaries in the mouse, and TAKEWAKI (1937) recorded a five-ova-containing follicle in the mouse. But they did not give any detailed morphology of polyovular follicles. DEDERER (1934), LANE (1938), and SIGEMORO (1947) reported the polyovular follicle in immature and mature rats. SIGEMORO (1947) made a detailed study with the Norway rat and reported that the total number of polyovular follicles in 120 mature rat ovaries from 60 individuals was found to be 375; among of them 316 follicles (84%) were biovular, triovular follicles were 47 (13%); 9 follicles (2%) contained four ova and 3 follicles (1%) included five ova. He described that the polyovular follicles were not less common in the rat than in other mammals.

In the present study working with mice, 70 to 120 days of age (mature mice), it has been revealed that the polyovular follicles is rather rare in occurrence; 80 polyovular follicles were found to occur in 60 ovaries under study. Of them, 62 follicles (78%) were biovular; 13 follicles (16%) were triovular; 4 follicles (5%) were tetraovular, 1 follicle (1%) included five ova (Table 2). When observed regarding the oestrous cycle, the ovaries from fourteen individuals contained 44 polyovular follicles at the proestrus stage, those from 5 individuals contained 9 at the oestrus stage, those from 7 individuals showed 18 at the metoestrus stage and those from 4 individuals included 9 at the dioestrus stage. The average number of the polyovular follicles observed in twenty-five individuals of S-strain mice was found

Table 1. Number of normal, abnormal mature follicles and polyovular follicles in normal mature ovaries of mice.

| Material | Oestrous cycle | Number of individual | Normal mature follicle | | | Abnormal mature follicle | | | Polyovular follicle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ovary | | Total | Ovary | | Total | Ovary | | Total |
| | | | Right | Left | | Right | Left | | Right | Left | |
| Thirty mature mice (*Mus musculus*, Siwss albinos) | Proestrus | 14 | 50 | 59 | 109 | 25 | 33 | 58 | 22 | 22 | 44 |
| | Oestrus | 5 | 10 | 10 | 20 | 7 | 8 | 15 | 4 | 5 | 9 |
| | Metoestrus | 7 | 15 | 13 | 28 | 4 | 4 | 8 | 13 | 5 | 18 |
| | Dioestrus | 4 | 0 | 0 | 0 | 3 | 1 | 4 | 4 | 5 | 9 |
| | Total | 30 | 75 | 82 | 157 | 39 | 46 | 85 | 43 | 37 | 80 |

Table 2. Type of abnormal mature follicles and polyovular follicles in normal mature ovaries of mice.

| Material | Oestrous cycle | Number of individual | Type of abnormal follicle | | | Type of polyovular follicle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Abnormality in granulosa cells | Abnormality in egg body | Total | Biovular | Triovular | Teraovular | Fiveovular | Total |
| Thirty mature mice (*Mus musculus*, Swiss albinos) | Proestrus | 14 | 49 | 9 | 58 | 33 | 7 | 3 | 1 | 44 |
| | Oestrus | 5 | 11 | 4 | 15 | 6 | 3 | 0 | 0 | 9 |
| | Metoestrus | 7 | 7 | 1 | 8 | 15 | 2 | 1 | 0 | 18 |
| | Dioestrus | 4 | 4 | 0 | 4 | 8 | 1 | 0 | 0 | 9 |
| | Total | 30 | 71 | 14 | 85 | 62 | 13 | 4 | 1 | 80 |

to be 2.7, with a range from 1 to 6.

The majority of the polyovular follicles was small in size with no antrum. It seems probable that the polyovular follicles are in the process of degeneration without establishing the antrum folliculi (Figs. 53-56). Only three biovular follicles were found in the stage of antrum formation. One of them showed an ovum advancing to the maturation division, while the other remained without the first maturation division (Figs. 63-65). There were also two other examples in which the first maturation spindles was formed in the ovum (Fig. 62). The evidence presented seems to suggest that polyovular follicles originate from fusion of the previously separated follicles, or arise through abnormal nuclear division, though there are a few polyovular follicles which resulted from the separation of an original ovum. They

seem to be the process of disintegration without advancing to the ovulation course.

Concerning the origin of polyovular follicles, HARTMAN (1926) enumerated three possible ways of formation; 1) by division of polyovular ova, 2) by concrescence of previously separated follicle, and 3) by persistent union of ova in the egg tube.

ISHIDA (1953) reported that the atretic ova of rat ovaries began to atrophy through three types of degeneration process. One type deals with that the ovum undergoes symmetrical cell division and then their cytoplasm degenerates showing irregular masses. He suggested that polyovular ova were in the process of degeneration without establishing the antrum folliculi as shown in Fig. 53. Recently, KENT (1959) informed that the occurrence of polyovular follicles and polynuclear ova were more common in immature animals than in mature ones, and he assumed that the feature may be due to alleviation of a deficient estrogen condition in young animals. The relation between the formation of polyovular follicles and the hormonal function in the ovary will be described in the next chapter.

According to the views of the present author, the polyovular follicles may be produced through the following three types of abnormal processes: 1) polyovular follicles may be formed by concresence of previously separated follicles, 2) polyovular ova may be produced by abnormal nuclear division, and 3) polyovular follicles may be arised by separation or fragmentation of the original ovum. Type 1 and 2 seem to be common in occurrence, though there are a few examples which are suggestive of the possibly of their normal ovulation course. But all ova of type 3 are doomed to atrophy in the ovary.

(4) Polyovular follicles in immature mice

The review of the extensive literature regarding polyovular follicles and polynuclear ova in the immature mammals was provided by LANE (1938), DAWSON (1951) and KENT (1959). The polyovular follicles were more common in occurrence in immature animals than in the mature individuals. LANE (1938) studied on the abnormal follicles in one hundred ovaries from sixty rats which varied in age from 15 to 64 days and reported that five follicles (0.018%) contained binuclear ova; thirteen follicles (0.045%) were biovular and three follicles (0.011%) were triovular. Only two of those atypical follicles had progressed in development to the stage of formation of the antrum. KENT (1959) investigated the polyovular follicles in young hamsters, 41 days of age, by treating them with estradiol monobenzoate in order to reduce the incidence of polyovular follicles and polynuclear ova. According to his results, the incidence of polyovular follicles showed a reduction of 18 percent.

The present study with immature mice, 10-20 days of age (7.0-9.0 gram in body weight), has made it clear that the polyovular follicles and polynuclear follicles are rather rare in occurrence. In 20 ovaries from a series of ten normal mice, 13 polyovular follicles and 1 nuclear ova were found in total. Of them, 10 follicles were biovular and 3 follicles were triovular (Table 3). All of them were in the process of atretic degeneration without establishment of the antrum folliculi. The

Table 3. Number of polyovular follicles from twenty ovaries of immature mice.

| Mouse Number | Body weight in gram | Right ovary | | | | Left ovary | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Biovular | Bi-nuclear | Tri-ovular | Tetra-ovular | Bi-ovular | Bi-nuclear | Tri-ovular | Tetra-ovular |
| No. 1 | 8.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| No. 2 | 8.0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| No. 3 | 8.5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| No. 4 | 9.0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| No. 5 | 7.0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| No. 6 | 8.0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| No. 7 | 8.5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| No. 8 | 8.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| No. 9 | 7.0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| No. 10 | 9.0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Total | | 5 | 1 | 0 | 0 | 5 | 0 | 0 | 3 |
| | | 6 | | | | 8 | | | |
| Average Number | | 14 | | | | | | | |

number of polyovular follicles in the immature mice was 1.5 in an average. On the other hand, mature mice, 70 to 120 days of age, weighting 25 to 28.5 gram, showed a value 2.7, with a range from 1 to 6. Generally, the polyovular follicles were found to be rather rare in occurrence in both mature and immature mice.

## Part II. Effects of sex stimulating hormones on abnormal follicles of mice

In the previous study, the author has made some cytological studies on abnormal mature follicles contained polyovular follicles in mouse ovaries during different phases of the oestrous cycle. Based on the results of observations, the suggestion may be possible that the disintegration may takes place in both the egg body and the granulosa cells under the influence of sex stimulating hormones on the follicular apparatus. Taking the above view in mind, the effects of sex stimulating hormones derived from the anterior pituitary gland, placenta gland and pregnant mare serum of horses, are to be examined in abnormal mature ova during the sexual cycle of mice in the present study.

LANE (1935 and 38) reported the influence of pituitary gonadotrophic hormones on the follicular apparatus of female rats which varied in age from 15 to 64 days. In his study of ovaries from animals treated with various hormone preparations (Aminiotin, F.S.H., L.H.), the conclusion was made that the influence of hormones on the atypical follicles materially altered under these experimental conditions. PINCUS and ENZMANN (1947) stated that the percentage of atretic ova in the rabbit young oocytes is low (10%). They indicated that among follicles of larger size about 60 percent were atretic at any one time, and that the hormone administration affected the largest follicles and their ova. PAYNE and HELLBAUM (1955) reported the possibility of a normal follicular maturation in the absence of the pituitary.

KENT (1959), in the young female golden-hamster, has pointed out that the reduction in number of polyovular follicles and polynuclear ova were obtained by treating with estradiol monobenzoate. The above feature seems to be due to allevation of a deficient estrogen condition in the young animal. However, no particular attention has been paid by any author on the influence of hormones on the abnormal follicles in mature animals.

There are many papers which deal with polyovulation under the effect of anterior pituitary hormones or pregnant mare serum or oestrogen in certain vertebrates (PINCUS 1943, ROBINSON 1949, GATES 1956, GREEN 1956, HUNTER, ADAM & ROBINSON 1955, RAESIDA & LAMOND 1956, OKIGAKI 1958, SATO 1959 and some others). The course of normal polyovulation under the influence of sex stimulating hormones has not yet been solved.

The following work was undertaken to dertermine whether or not the administration of anterior pituitary sex gland stimulating hormones, and the sex gland stimulating hormones of placenta and pregnant mare serum of horses would reduce the number of abnormal mature ova and polyovular ova in mature female mice.

(1) Effects of mixture of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormones of placenta.

Twenty-five mice received the injection of mixed hormones "Synaphorin"[1] (Teikoku Hormone MFG. Co.) had ovaries which were heavily weighted than normal ovaries. It was reported that "Synaphorin" contains follicle stimulating as well as luteinizing hormones, and stimulates the growth of primary and secondary sex glands of both sexes, or engage in the up-keep of their functions.

Microscopical observations have revealed that the abnormal mature ova proceeding beyond the maturation stage are smaller in number in hormone treated mice than in the untreated mice. The atretic follicles of larger size which advance towards the maturation course were very abundant in number in the untreated mature ovaries as shown in Part I. Thirty untreated individuals were found to have in total 157 normal mature ova, 85 abnormal mature ova, and 80 polyovular ova at four stages of the oestrous cycle. Fourteen individuals observed at the proestrus stage were found to possess 109 normal, 58 abnormal, and 44 polyovular ova. Five individuals at the oestrus stage were found to have 20 normal, 15 abnormal, and 9 polyovular ova. Seven individuals at the metoestrus stage showed 28 normal, 8 abnormal, and 18 polyovular ova. Four individuals at the dioestrus stage were observed to have no normal, 4 abnormal, and 9 polyovular ova.

On the other hand, twenty-five mice which received the injection of "Synaphorin" showed in their ovaries 202 normal mature ova, 45 abnormal mature ova, and 48 polyovular ova in total. Seventeen individuals at the proestrus stage were observed to have 156 normal, 30 abnormal, and 29 polyovular ova. Two individuals at the oestrus stage possessed 19 normal, 2 abnormal, and 5 polyovular ova. Four individuals at the metoestrus stage were found to contain 20 normal, 10 ab-

1) "Synaphrin" is a mixture of two kinds of hormones: a sex gland stimulating hormone taken from the anterior pituitary gland and a sex stiumulating hormone taken from human placenta.

normal, and 5 polyovular ova. Three individuals at the dioestrus stage showed 7 normal, 3 abnormal, and 9 polyovular ova (Table 4).

The control data from 17 untreated mouse ovaries, showed 144 normal, 83 abnormal, and 37 polyovular ova in total. Five individuals at the proestrus stage were formed to have 56 normal, 26 abnormal, and 16 polyovular ova. Five individuals at the oestrus stage were observed to have 30 normal, 19 abnormal, and 8 polyovular ova. Two individuals at the metoestrus stage were found to show 9 normal, 8 abnormal, and 2 polyovular ova. Five individuals at the late dioestrus stage were observed to present 49 normal, 30 abnormal, and 11 polyovular ova at the oestrus stages (Table 8).

Abnormal mature ova showed the chromosomes laying on the equatorial plate of the first spindle, having no nuclear membrane and antrum. Some ova showed the second polar spindle at metaphase accompanied by the first polar body in the follicle with a little antra. Some others showed the completion of the second division offering the second polar body, though no antrum follicle has been formed. There occur some chromosome abnormalities and the abberant first division showing a terapolar spindle. In the extreme case, the ovum contained many vacuoles in its ooplasm. The abnormal mature ova were abundant in number in untreated ovaries. The evidence here presented indicated that the abnormal ova decreased in number in treated ovaries in comparison with those in untreated ovaries, probably under the influence of sex stimulating hormones on mature ova.

In the atretic ova which showed advance towards the maturation division, the disintegration took place either in the granulosa cells or in the egg body itself in the process of degeneration. Thirty untreated mice which showed the former type of abnormal ova were 71 in number and those of the latter type were 14 in total as reported in the Part I (Table 2).

Twenty-five mice treated with the anterior pituitary hormone (A.P.H.) showed 35 abnormal ova of the former type, 10 such ova of the latter type in total (Table 5). Seventeen control mice showed 71 abnormal ova of the former type, and 12 of the latter type (Table 8).

From the results of the above experiments, it is evident that the decrease in number of abnormal ova showing immature condition of granulosa cells is remarkable, and further that in the A.P.H. treated mice the reduction of polyovular ova was not striking. The results of the present study with mice, 70 to 120 days of age has shown that polyovular ova were rather rare in occurrence in a normal condition. Out of them, 32 follicles (67%) contained biovular, 15 follicles (31%) were triovular, and 1 follicle (2%) was tetraovular. Sixteen individuals observed at the proestrus stage showed 20 biovular, 8 triovular and 1 tetraovular, 2 individuals at the oestrus stage were found to show 4 biovular and 1 triovular, 4 individuals at the metoestrus stage showed 3 biovular and 2 triovular, and 3 individuals at the late dioestrus stage were observed to possess 5 biovular and 4 triovular (Table 5). The majority of polyovular follicles (92%) were in the process of degeneration, though there were four examples of biovular follicles (8%) which are suggestive of

Table 4. Number of normal, abnormal mature follicles and polyovular follicles in A.P.H. treated mouse ovaries.

| Material | Oestrous cycle | Number of individual | Normal follicle | | | Abnormal follicle | | | Polyovular follicle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ovary | | Total | Ovary | | Total | Ovary | | Total |
| | | | Right | Left | | Right | Left | | Right | Left | |
| Twenty-five mature mice (*Mus musculus*, "Swiss albinos") | Proestrus | 16 | 89 | 67 | 156 | 11 | 19 | 30 | 18 | 11 | 29 |
| | Oestrus | 2 | 9 | 10 | 19 | 1 | 1 | 2 | 3 | 2 | 5 |
| | Metoestrus | 4 | 9 | 11 | 20 | 8 | 2 | 10 | 3 | 2 | 5 |
| | Late Dioestrus | 3 | 3 | 4 | 7 | 2 | 1 | 3 | 4 | 5 | 9 |
| | Total | 25 | 110 | 92 | 202 | 22 | 23 | 45 | 28 | 20 | 48 |

Table 5. Type of abnormal mature follicles and polyovular follicles in A.P.H. treated mouse ovaries.

| Material | Oestrous cycle | Number of individual | Type of abnormal follicle | | | Type of polyovular follicle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Abnormality in granulosa cells | Abnormality in egg body | Total | Biovular | Triovular | Tetraovular | Fiveovular | Total |
| Twenty-five mature mice (*Mus musculus*, "Swiss albinos") | Proestrus | 16 | 24 | 6 | 30 | 20 | 8 | 1 | 0 | 29 |
| | Oestrus | 2 | 1 | 1 | 2 | 4 | 1 | 0 | 0 | 5 |
| | Metoestrus | 4 | 7 | 3 | 10 | 3 | 2 | 0 | 0 | 5 |
| | Late Dioestrus | 3 | 3 | 0 | 3 | 5 | 4 | 0 | 0 | 9 |
| | Total | 25 | 35 | 10 | 45 | 32 | 15 | 1 | 0 | 48 |

the possibility of their maturation.

Seventeen control mice in this experiment showed 28 follicles (76%) which contained biovular, 4 follicles (11%) which showed triovular, 3 follicles (8%) which were tetraovular, and 2 follicles (5%) which contained five-ovular ova (Table 9).

The data obtained from this observation, have shown that the polyovular ova were small in average number in A.P.H. treated mice than in the untreated mice. It was rather difficult to determine whether or not the administration of anterior pituitary hormone and the sex gland stimulating hormones of placenta would reduce the number of polyovular follicles (Text-figs. 1-2).

(2) Effects of the mixture of the anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormone from placenta and pregnant mare

serum of horses.

The following experiments were undertaken in order to determine whether or not the administration of pregnant mare serum of horses (P.M.S.) would reduce the number of abnormal mature ova and polyovular follicles in mouse ovaries than in A.P.H. treated ovaries.

It was found that the ovaries of 25 P.M.S. treated mice were more heavily weighted than normal untreated ovaries. The hormone used here as P.M.S. was "Synaphorin" (Teikoku Hormone MFG., Co.). The results of the application of Synaphorin indicated that abnormal ova proceeding further beyond the maturation division were very small in number in P.M.S. treated mice than in untreated mice. Twenty-five treated mice showed in their ovaries 308 normal mature ova, 46 abnormal mature ova, and 32 polyovular ova in total. Nine individuals examined at the proestrus stage showed 162 normal, 22 abnormal, and 16 polyovular ova. Seven individuals at the oestrus stage contained 93 normal, 11 abnormal, and 8 polyovular ova. Four individuals at the metoestrus stage were found to have 25 normal, 10 abnormal, and 3 polyovular ova. Five individuals at the late dioestrus and proestrus stage were observed to show 28 normal, 3 abnormal, and 5 polyovular ova (Table 6).

Seventeen mice which reserved as control were observed to possess 144 normal, 83 abnormal, and 37 polyovular ova in total. The data here obtained indicated a remarkable reduction in number of abnormal ova in P.M.S. treated mice than in A.P.H. treated mice (Text-figs. 1-2). Particulary, the decrease in number of abnormal mature ova showing an immature condition of granulosa cells is remarkable.

Abnormal mature ova observed in 25 P.M.S. treated mice showed a disintegration taking place either in the granulosa cell or in the egg body itself. The abnormality of the former type were found in 39 examples, that of the latter type were in 7 examples in total. Seventeen control mice showed 71 abnormal ova of the former type and 12 ova of the latter type (Table 7).

In the polyovular ova observed here, 25 follicles (78%) contained biovular ova, 6 follicles (19%) contained triovular ones, and 1 follicle (3%) showed five ova. The majority of ova (91%) were in the process of degeneration, though there were 3 examples of biovular follicles (9%) which were suggestive of the possibility of their maturation.

Based on the results of the present study, it is evident that the polyovular follicles slightly decreased in number in P.M.S. treated mice as compared with untreated mice. It is difficult to state from the above data that the hormones affected the reduction of polyovular follicles.

On the basis of the results of the present observations, the conclusion is possible that the reduction in number of abnormal mature ova may be explicable by the effect of sex stimulating hormones. Also the evidence presented in the two experiments mentioned above has indicated that an abnormal mature ova showed a remarkable decrease in number in P.M.S. treated mice in comparison with A.P.H.

Table 6. Number of normal, abnormal mature follicles and polyovular follicles in P.M.S. treated mouse ovaries.

| Material | Oestrous cycle | Number of individual | Normal follicle | | | Abnormal follicle | | | Polyovular follicle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ovary | | Total | Ovary | | Total | Ovary | | Total |
| | | | Right | Left | | Right | Left | | Right | Left | |
| Twenty-five mature mice (*Mus musculus*, "Swiss albinos") | Proestrus | 9 | 88 | 74 | 162 | 11 | 11 | 22 | 8 | 8 | 16 |
| | Oestrus | 7 | 45 | 48 | 93 | 6 | 5 | 11 | 5 | 3 | 8 |
| | Metoestrus | 4 | 12 | 13 | 25 | 6 | 4 | 10 | 2 | 1 | 3 |
| | Late Dioestrus | 5 | 12 | 16 | 28 | 1 | 2 | 3 | 1 | 4 | 5 |
| | Total | 25 | 157 | 151 | 308 | 24 | 22 | 46 | 16 | 16 | 32 |

Table 7. Type of abnormal mature follicles and polyovular follicles in P.M.S. treated mouse ovaries.

| Material | Oestrous cycle | Number of individual | Type of abnormal follicle | | | Type of polyovular follicle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Abnormality in granulosa cells | Abnormality in egg body | Total | Biovular | Triovular | Tetraovular | Fiveovular | Total |
| Twenty-five mature mice (*Mus musculus*, "Swiss albinos") | Proestrus | 16 | 19 | 3 | 22 | 10 | 5 | 0 | 1 | 16 |
| | Oestrus | 2 | 9 | 2 | 11 | 7 | 1 | 0 | 0 | 8 |
| | Metoestrus | 4 | 9 | 1 | 10 | 3 | 0 | 0 | 0 | 3 |
| | Late Dioestrus | 3 | 2 | 1 | 3 | 5 | 0 | 0 | 0 | 5 |
| | Total | 25 | 39 | 7 | 46 | 25 | 6 | 0 | 1 | 32 |

treated mice (Text-figs. 1-2).

Recently many papers have appeared regarding polyovulation under the effect of anterior pituitary hormone or oestrogen or pregnant mare serum in certain vertebrates. RUNNER & PALM (1953) observed artificial polyovulation in immature mice which received injections of cholionic gonadotropin and pregnant mare serum gonadotropin. Also, PINCUS (1940), GATES (1956), GREEN (1956) observed polyovulation in mature mice which received injections of the anterior pituitary gonadotrophic hormone. SATO (1958) and OKIGAKI (1959) published the results of preliminary observations on hormone induced polyovulation and superfecundity in mature mice of D-240 strain which treated with the anterior pituitary gonadotrophic hormone and pregnant mare serum gonadotropin. ROBINSON (1949), RASEIDA &

Table 8. Number of normal, abnormal mature follicles and polyovular follicles in control mouse ovaries.

| Material | Oestrous cycle | Number of individual | Normal follicle | | | Abnormal follicle | | | Polyovular follicle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ovary | | Total | Ovary | | Total | Ovary | | Total |
| | | | Right | Left | | Right | Left | | Right | Left | |
| Seventeen mature mice (*Mus musculus*. "Swiss albinos") | Proestrus | 5 | 23 | 33 | 56 | 11 | 15 | 26 | 10 | 6 | 16 |
| | Oestrus | 5 | 16 | 14 | 30 | 9 | 10 | 19 | 6 | 2 | 8 |
| | Metoestrus | 2 | 5 | 4 | 9 | 5 | 3 | 8 | 1 | 1 | 2 |
| | Late Dioestrus | 5 | 21 | 28 | 49 | 18 | 12 | 30 | 6 | 5 | 11 |
| | Total | 17 | 65 | 79 | 144 | 43 | 40 | 83 | 23 | 14 | 37 |

Table 9. Type of abnormal mature follicles and polyovular follicles in control mouse ovaries.

| Material | Oestrous cycle | Number of individual | Type of abnormal follicle | | | Type of polyovular follicle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Abnormality in granulosa cells | Abnormality in egg body | Total | Biovular | Tri-ovular | Tetra-ovular | Five-ovular | Total |
| Seventeen mature mice (*Mus musculus*, Swiss albinos) | Proestrus | 5 | 23 | 3 | 26 | 13 | 0 | 2 | 1 | 16 |
| | Oestrus | 5 | 16 | 3 | 19 | 6 | 2 | 0 | 0 | 8 |
| | Metoestrus | 2 | 5 | 3 | 8 | 1 | 0 | 1 | 0 | 2 |
| | Late Dioestrus | 5 | 27 | 3 | 30 | 8 | 2 | 0 | 1 | 11 |
| | Total | 17 | 71 | 12 | 83 | 28 | 4 | 3 | 2 | 37 |

LAMOND (1956) and some others recorded the effect of estrogen and pregnant mare serum of horses in higher vertebrates. According to them, the single application of P.M.S. produced no effective on polyovulation. SATO (1959) has made some observation on hormone induced polyovulation and superfecundity in mature mice, and reported that the number of eggs ovulated was 23 to 135 in treated mice, being 58.5 on an average. The number of eggs at the natural ovulation of mice was 8.2 on an average. OKIGAKI (1959) observed natural and artificial ovulation in mature and immature rats, and reported that one mature rat discharged 40 eggs, 31 from the left ovary and 9 from the right ovary through the injection of the gonadotrophic hormone. Control untreated rats observed ovulated 8 to 10 eggs in the majority of cases, being 8.8 on an average.

The data obtained in the present study are presented in Table 10 in which the average numbers of normal ova, abnormal ova and polyovular ova in normal and hormone treated mice are given. In untreated ovaries 5.2 normal ova, 2.8 abnormal ova, and 2.7 polyovular ova were observed, while A.P.H. treated mice showed 8.1 normal ova, 1.8 abnormal ova, and 1.9 polyovular ova, and P.M.S. treated mice presented 12.3 normal ova, 1.8 abnormal ova, and 1.3 polyovular ova. Untreated control mice furnished 8.5 normal, 4.9 abnormal, and 2.2 polyovular ova.

From the results of this study, the conclusion is possible that the reduction in number of abnormal mature ova may be explicable by the effect of sex stimulating hormones. Also, the evidence presented in the above two experiments has indicated that the abnormal ova significantly decrease in number in P.M.S. treated mice in comparison with A.P.H. treated mice. It is presumed that abnormal mature ova which failed to ovulate may undergo ovulation after their growth under the influence of sex stimulating hormones, and that the hormones seem not to affect upon atretic ova in young oocytes or in primary follicles. Further, the results of observations indicated that the hormones did not affect the reduction of polyovular follicles, so far as the present experiments have gone in mature mice.

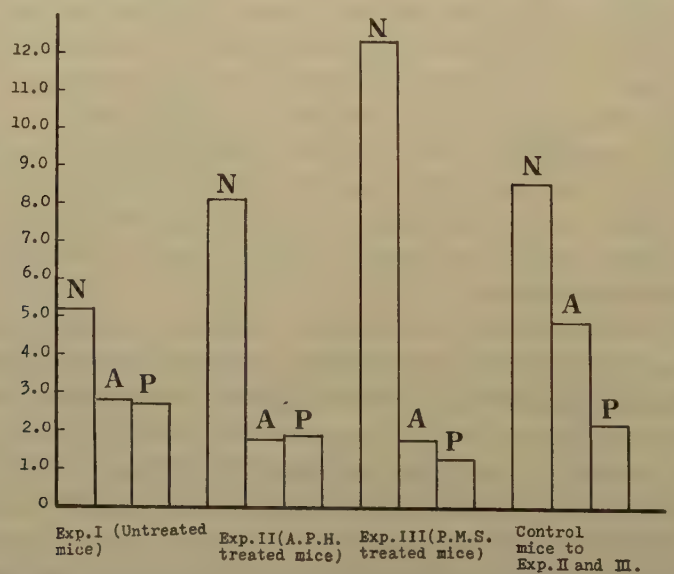
The number of polyovular follicles was observed in Swiss albinos showing that the polyovular follicles are rather rare in occurrence. The number of polyovular follicles was studied in A.P.H. treated and non- treated Swiss albinos. The untreated mice showed 2.7 polyovular ova in average, while treated mice showed 1.9 polyovular ova. In P.M.S. treated mice, polyovular ova were 1.3. From the above findings it can be stated that the hormone treated mice indicate a slight decrease in number of polyovular ova, but the effect of hormones is not remarkable on the reduction in number of polyovular follicles. KENT (1959) reported the reduction of polyovular follicles and polynuclear ova following the application of estradiol monobenzoate (Estrogen) in the immature golden hamster. According to him, polyovular follicles and polynuclear ova reduced 17 to 18 percent as compared with control value. Generally, immature mammalian animals were found to have many polyovular follicles and polynuclear ova, while in mice the polyovular follicles are rather rare in occurrence. Taken into consideration the above fact, the effect of the sex stimulating hormone on polyovular follicles may not be conclusive in the present status of investigation.

PINCUS & ENZMANN (1947) assumed that maturation of atretic ovarian eggs in the rabbit ovary, was found in small atresia of young oocytes (10%), and that the larger follicles at 60 percent were found to be atretic. According to them, the hormone administration affected only the largest follicle and their contained ova.

From the results of observations described in Part I and II, it is apparent that the atretic follicles of larger size in which the ova showed advance towards the maturation course are very abundant in number in ovaries of mature mice, being 35% in total. It is most probable that the atretic follicles are in the process of degeneration, and that the disintegration takes place either in the egg body or in the granulosa cells. From this evident it is presumable that there is a relationship between the

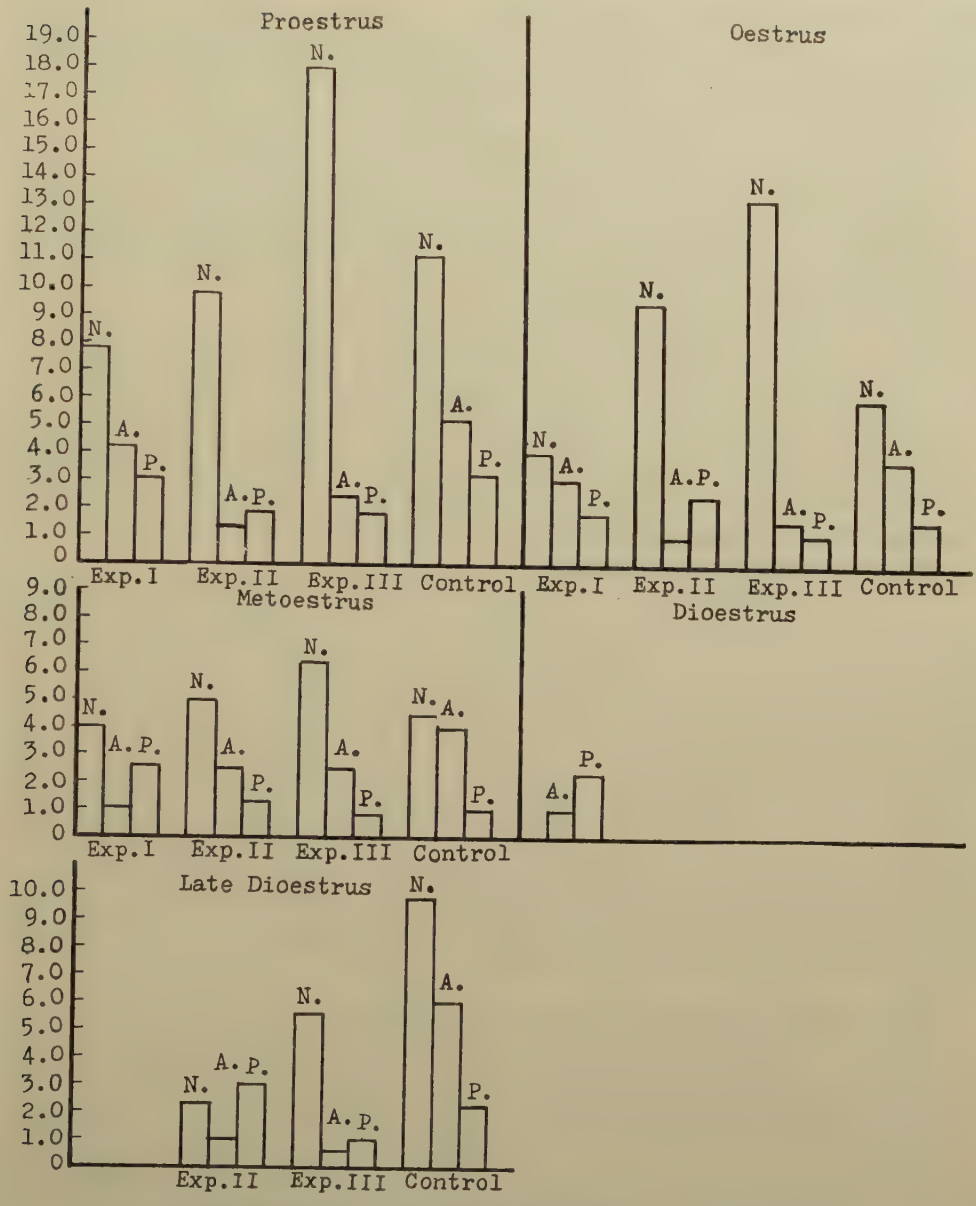
Table 10. Average number of normal, abnormal mature follicles and polyovular follicles in mouse ovaries at three experiments.

| Method | Oestrous cycle | Number of individual | Average number of normal follicle | Average number of abnormal follicle | Average number of polyovular follicle |
|---|---|---|---|---|---|
| Exp. I (Untreated mice) | Proestrus | 14 | 7.8 | 4.1 | 3.1 |
| | Oestrus | 5 | 4.0 | 3.0 | 1.8 |
| | Metoestrus | 7 | 4.0 | 1.1 | 2.6 |
| | Dioestrus | 4 | 0 | 1.0 | 2.3 |
| | Total | 30 | 5.2 | 2.8 | 2.7 |
| Exp. II (A.P.H. treated mice) | Proestrus | 16 | 9.8 | 1.3 | 1.8 |
| | Oestrus | 2 | 9.5 | 1.0 | 2.5 |
| | Metoestrus | 4 | 5.0 | 2.5 | 1.3 |
| | Late Dioestrus | 3 | 2.3 | 1.0 | 3.0 |
| | Total | 25 | 8.1 | 1.8 | 1.9 |
| Exp. III (P.M.S. treated mice) | Proestrus | 9 | 18.0 | 2.4 | 1.8 |
| | Oestrus | 7 | 13.3 | 1.6 | 1.1 |
| | Metoestrus | 4 | 6.3 | 2.5 | 0.8 |
| | Late Dioestrus | 5 | 5.6 | 0.6 | 1.0 |
| | Total | 25 | 12.3 | 1.8 | 1.3 |
| Control (Exp. II and Exp. III) | Proestrus | 5 | 11.2 | 5.2 | 3.2 |
| | Oestrus | 5 | 6.0 | 3.8 | 1.6 |
| | Metoestrus | 2 | 4.5 | 4.0 | 1.0 |
| | Late Dioestrus | 5 | 9.8 | 6.0 | 2.2 |
| | Total | 17 | 8.5 | 4.9 | 2.2 |




N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 1. Average number of normal, abnormal mature follicles and polyovular follicles in the mouse ovaries at three experiments.




N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 2. Average number of normal, abnormal mature follicles and polyovular follicles in mice taken at different phases of the oestrous cycle.

maturation of the Graafian follicles and the effects of the sex stimulating hormone in the largest follicle: the abnormal ova preceding further beyond the maturation course are very few in number in hormone treated mice than in the untreated mice. Especially, P.M.S. treated mice indictated the remarkable decrease in number of abnormal ova. Further, the decrease in number of abnormal ova showing an im-

mature condition of grnulosa cells is remarkable. Based on the results of the present study the assumption is possibly made that abnormal mature ova which failed to ovulate may undergo ovulation under the effect of the sex stimulating hormones.

Part III. Number of Aberrant follicles in relation to the strain of mice

The above investigations have dealt with cytological studies carried out with the ova derived from 66 ovaries which were taken from a series of mature unmated mice of Swiss-albinos at different phases of the oestrous cycle, with special reference to the maturation and degeneration ova in mature ovaries.

Part III is devoted to the cytological research on the number of aberrant follicles in relation to mouse strain. The strains of mice used here are Swiss albinos, D-240 albinos, dd/Ma albinos and B-72 albinos. They are 70 to 120 days of age, weighting about 20 to 30 gm. The work was carried out during from late summer to late autumn through active breeding seasons in the year. The work was aimed to determine whether or not the four mouse strains are different in the number of normal, abnormal and polyovular ova.

The full grown ovarian egg containing the resting nucleus is approximately spherical in shape and lies in an eccentric position in the Graafian follicle. Such a mature follicle is found occupying a position at the periphery of the ovary. The ovum is surrounded by the zona pellucida and the latter again by the corona radiata. Surrounding the corona radiata there are several layers of cells, stratum granulosum which are arranged lining surface of the follicle and enclosing a huge cavity filled up with the fluid, liquor folliculi. The size of the mouse egg are from 51 to 82$\mu$ in S-strain, 75-82$\mu$ in dd/Ma strain, 78-89$\mu$ in B-72 strain, 73-80$\mu$ in D-strain mice under the fixed condition. The mature follicle are 217-290$\mu$ in diameter in S-strain, 286–340$\mu$ in B-72 strain, 286–350$\mu$ in dd/Ma strain, 243–357$\mu$ in D-strain in diameter. The size of the mouse egg therefore nearly approximates in four different strains.

Microscopical observations revealed that the normal and abnormal mature ova proceeding prior to the maturation course and polyovular ova are abundant in four strains of mice. Swiss albinos showed in their mature ovaries 157 normal mature ova, 85 abnormal mature ova and 80 polyovular ova in total. Fourteen individuals observed at the proestrus stage showed 109 normal, 58 abnormal, and 44 polyovular follicles. Five individuals at the oestrus stage indicated 20 normal, 15 abnormal, and 9 polyovular ones. Seven individuals at the metoestrus stage presented 28 normal, 8 abnormal, and 18 polyovular ones. Four individuals at the dioestrus stage showed no normal, 4 abnormal, and 9 polyovular follicles.

D-240 mice showed in their mature ovaries from 30 individuals showed 129 normal, 86 abnormal, and 56 polyovular follicles in total. Six individuals at the proestrus stage presented 43 normal, 19 abnormal, and 10 polyovular ones. Seven individuals at oestrus stage showed 58 normal, 36 abnormal, and 11 polyovular ones. Eighteen individuals at the metoestrus stage indicated 26 normal, 19 abnormal, and 14 polyovular ones. Seven individuals at the dioestrus stage showed no normal, 9

abnormal, and 17 polyovular ones. Two individuals at the late dioestrus stage provided 2 normal, 3 abnormal, and 2 polyovular follicles.

dd/Ma mice furnished in their mature ovaries from twenty specimens 118 normal, 69 abnormal, and 36 polyovular follicles in total. Five individuals at the proestrus stage showed 26 normal, 15 abnormal, and 8 polyovular follicles. Six individuals at the oestrus stage indicated 58 normal, 23 abnormal, and 17 polyovular ones. Four individuals at the metoestrus stage presented 25 normal, 14 abnormal, and 5 polyovular ones. One individual at the dioestrus stage showed no normal, 2 abnormal, and no polyovular ones. Four individuals at the late dioestrus stage furnished 9 normal, 15 abnormal, and 6 polyovular follicles.

B-72 mice showed in their mature ovaries from twenty specimens 87 normal, 37 abnormal, and 20 polyovular follicles in total. Four individuals at the proestrus stage showed 24 normal, 14 abnormal, and 5 polyovular ones. Six individuals at the oestrus stage indiacted 42 normal, 12 abnormal, and 7 polyovular ones. One individual at the metoestrus stage presented 5 normal, 3 abnormal, and 2 polyovular ones. Four individuals at the dioestrus stage showed no normal, 3 abnormal, and 4 polyovular ones. Five individuals at the late dioestrus stage provided 16 normal, 5 abnormal, and 2 polyovular follicles (Table 11).

In abnormal mature ova, the disintegration took place either in the granulosa cells or in the egg body itself. In D-strain mice, the abnormal ova of the former type were found in 82 examples, those of the latter type being observed in 4 examples in total. In dd/Ma mice the former type ova were 60 and the latter type ones were 9 in total. In B-72 mice, the former type ova were 33 and the latter type ones were 4. The results from S-strain mice are given in Part I.

Next, the average number of normal, abnormal and polyovular ova were observed in four mouse strains. In S-strain mice the average numbers were 5.2 for normal, 2.8 for abnormal, and 2.7 for polyovular ova. In D-strain mice, they were 4.3 for normal, 2.9 for abnormal, and 1.8 for polyovular ova. In dd/Ma mice, they were 5.9 for normal, 3.5 for abnormal, and 1.8 for polyovular ova. In B-72 mice, they were 4.4 for normal, 1.9 abnormal, and 1.0 polyovular ova. The data obtained for different stage of the oestrous cycle are given in Table 12.

Based on the data obtained from the above observations, it is evident that the number of normal and abnormal mature ova showed no remarkable difference in four strains of mice.

The polyovular follicles were observed in four strains of mice, 70 to 120 days of age. The results revealed that the polyovular follicles were rather rare in occurrence and not different by strain.

In S-strain mice 80 polyovular follicles were found to occur in 60 ovaries. Of them, they were 62 follicles (78%) which were biovular, 13 follicles (16%) which were triovular, and 5 follicles (6%) which were tetraovular. Only three biovular follicles were found in the stage of normal antrum formation, and the other follicles are in the process of degeneration without the establishment to the antrum follicle.

In D-strain mice 54 polyovular follicles were found to occur in 60 ovaries. Of

them, there were 42 follicles (78%) which were biovular, 7 follicles (13%) which were triovular, and 5 follicles (9%) which were tetraovular. All are the atretic follicles with no antrum.

In dd/Ma mice 36 polyovular follicles were found to occur in 40 ovaries. Of them, there were 28 follicles (78%) which were biovular, 3 follicles (8%) which were triovular, 3 follicles (8%) which were tetraovular and 2 follicles (6%) which contained five ova. Two biovular follicles were found in the stage of the normal antrum formation and one of them seems to suggest that biovular follicles originate from the previously separated follicles and other follicles seem to arise through abnormal nuclear division in the second maturation division.

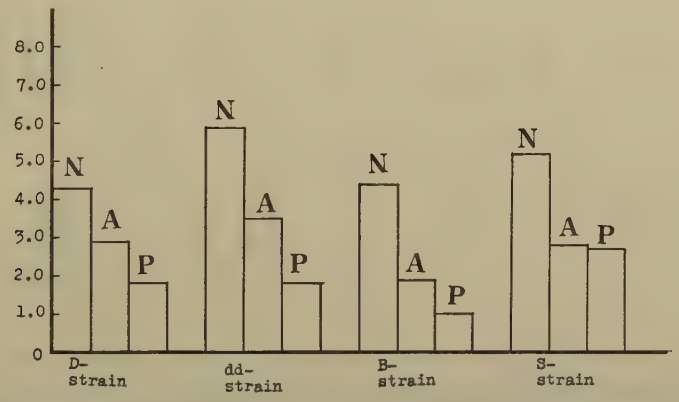
In B-strain mice, 20 polyovular follicles were found to occur in 40 ovaries. Of them, there were 18 follicles (90%) which were biovular, and 2 follicles (10%)

Table 11. Number of normal, abnormal mature follicles in mature ovaries at three mouse strains.

| Material | Oestrous cycle | Number of individual | Normal follicle | | | Abnormal follicle | | | Polyovular follicle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ovary | | Total | Ovary | | Total | Ovary | | Total |
| | | | Right | Left | | Right | Left | | Right | Left | |
| D-240 albinos strain | Proestrus | 6 | 18 | 25 | 43 | 8 | 11 | 19 | 5 | 5 | 10 |
| | Oestrus | 7 | 31 | 27 | 58 | 20 | 16 | 36 | 6 | 5 | 11 |
| | Metoestrus | 8 | 13 | 13 | 26 | 10 | 9 | 19 | 9 | 5 | 14 |
| | Dioestrus | 7 | 0 | 0 | 0 | 5 | 4 | 9 | 10 | 7 | 17 |
| | Late Dioestrus | 2 | 0 | 2 | 2 | 1 | 2 | 3 | 1 | 1 | 2 |
| | Total | 30 | 62 | 67 | 129 | 44 | 42 | 86 | 31 | 23 | 54 |
| dd/Ma albinos strain | Proestrus | 5 | 15 | 11 | 26 | 9 | 6 | 15 | 7 | 1 | 8 |
| | Oestrus | 6 | 26 | 32 | 58 | 13 | 10 | 23 | 8 | 9 | 17 |
| | Metoestrus | 4 | 15 | 10 | 25 | 11 | 3 | 14 | 3 | 2 | 5 |
| | Dioestrus | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 |
| | Late Dioestrus | 4 | 3 | 6 | 9 | 7 | 8 | 15 | 4 | 2 | 6 |
| | Total | 20 | 59 | 59 | 118 | 42 | 27 | 69 | 22 | 14 | 36 |
| B-72 albinos strain | Proestrus | 4 | 13 | 11 | 24 | 5 | 9 | 14 | 2 | 3 | 5 |
| | Oestrus | 6 | 26 | 16 | 42 | 6 | 6 | 12 | 5 | 2 | 7 |
| | Metoestrus | 1 | 2 | 3 | 5 | 3 | 0 | 3 | 1 | 1 | 2 |
| | Dioestrus | 4 | 0 | 0 | 0 | 2 | 1 | 3 | 2 | 2 | 4 |
| | Late Dioestrus | 5 | 7 | 9 | 16 | 1 | 4 | 5 | 1 | 1 | 2 |
| | Total | 20 | 48 | 39 | 87 | 17 | 20 | 37 | 11 | 9 | 20 |

Table 12. Average number of normal, abnormal mature follicles and polyovular follicles in mature ovaries at three mouse strains.

| Method | Oestrous cycle | Number of individual | Average number of normal follicle | Average number of abnormal follicle | Average number of polyovular follicle |
|---|---|---|---|---|---|
| D-240 albino strain | Proestrus | 6 | 7.2 | 3.2 | 1.7 |
| | Oestrus | 7 | 8.3 | 5.1 | 1.6 |
| | Metoestrus | 8 | 3.3 | 2.4 | 1.8 |
| | Dioestrus | 7 | 0 | 1.3 | 2.4 |
| | Late Dioestrus | 2 | 1.0 | 1.5 | 1.0 |
| | Total | 30 | 4.3 | 2.9 | 1.8 |
| dd/Ma albino strain | Proestrus | 5 | 5.2 | 3.0 | 1.6 |
| | Oestrus | 6 | 9.7 | 3.8 | 2.8 |
| | Metoestrus | 4 | 6.3 | 3.5 | 1.3 |
| | Dioestrus | 1 | 0 | 2.0 | 0 |
| | Late Dioestrus | 4 | 2.3 | 3.8 | 1.5 |
| | Total | 20 | 5.9 | 3.5 | 1.8 |
| B-72 albino strain | Proestrus | 4 | 6.0 | 3.5 | 1.3 |
| | Oestrus | 6 | 7.0 | 2.0 | 1.2 |
| | Metoestrus | 1 | 5.0 | 3.0 | 2.0 |
| | Dioestrus | 4 | 0 | 0.8 | 1.0 |
| | Dioestrus Late | 5 | 3.2 | 1.0 | 0.4 |
| | Total | 20 | 4.4 | 6.1 | 1.0 |




N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 3. Average number of normal, abnormal mature follicles and polyovular follicles in the mouse ovaries at four strains.

which were tetraovular. All of them are in the process of degeneration without establishing the antrum follicle (Table 13).

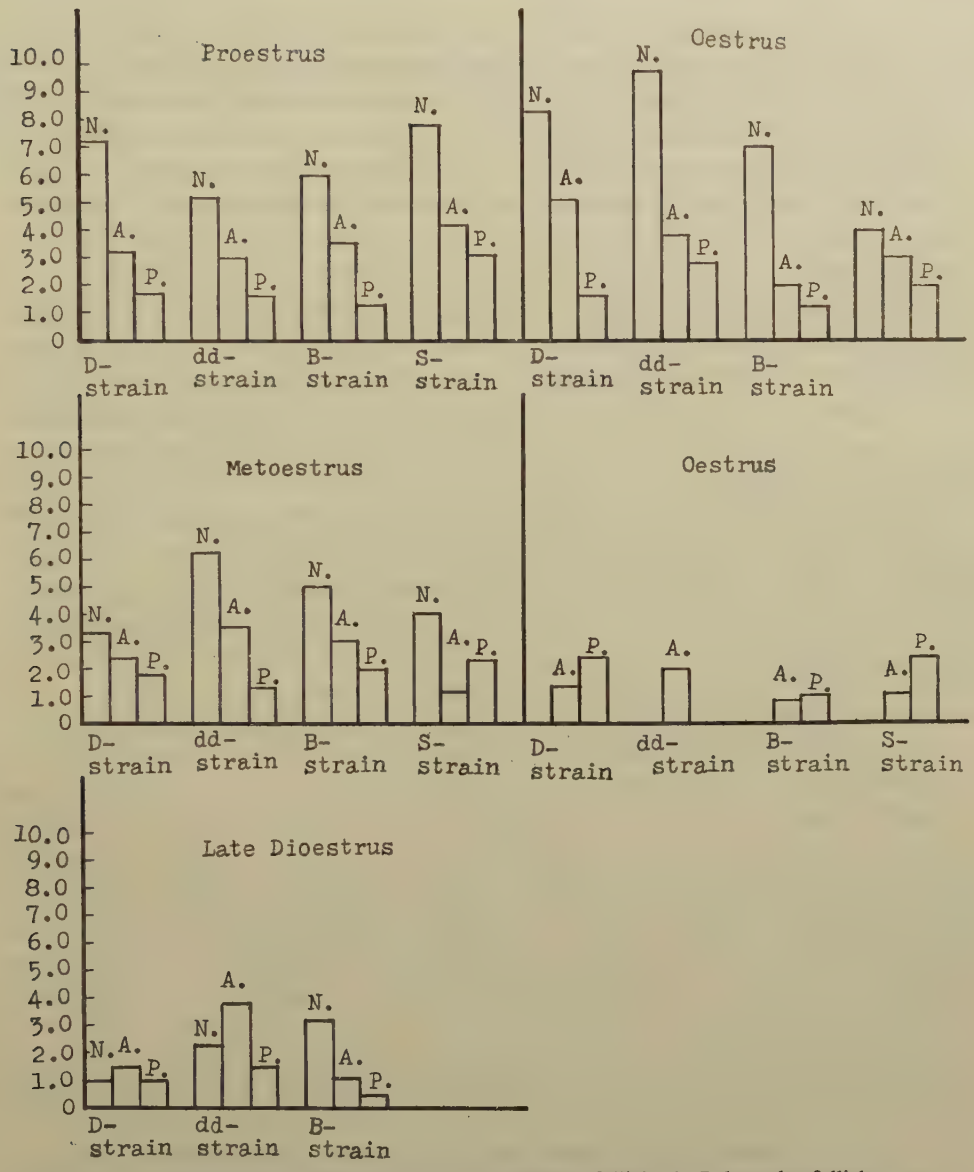
The data herein presented were based on observations with the ovaries derived from four mouse strains: they show no remarkable difference in number of normal, abnormal mature ova and polyovular ova by mouse strain. The features above

Table 13. Type of abnormal mature follicles and polyovular follicles in mature ovaries at three mouse strains.

| Material | Oestrous cycle | Type of abnormal follicle | | | Type of polyovular follicle | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Abnormality in granulosa cells | Abnormality in egg body | Total | Biovular | Tri-ovular | Tetra-ovular | Five-ovular | Total |
| D-240 albinos strain | Proestrus | 19 | 0 | 19 | 8 | 1 | 1 | 0 | 10 |
| | Oestrus | 35 | 1 | 36 | 9 | 2 | 1 | 0 | 11 |
| | Metoestrus | 19 | 0 | 19 | 12 | 1 | 0 | 0 | 14 |
| | Dioestrus | 6 | 3 | 9 | 11 | 3 | 3 | 0 | 17 |
| | Late Dioestrus | 3 | 0 | 3 | 2 | 0 | 0 | 0 | 2 |
| | Total | 82 | 4 | 86 | 42 | 7 | 5 | 0 | 54 |
| dd/Ma albinos strain | Proestrus | 13 | 2 | 15 | 6 | 0 | 1 | 1 | 8 |
| | Oestrus | 19 | 4 | 23 | 13 | 2 | 1 | 1 | 17 |
| | Metoestrus | 11 | 3 | 14 | 3 | 1 | 1 | 0 | 5 |
| | Dioestrus | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| | Late Dioestrus | 15 | 0 | 15 | 6 | 0 | 0 | 0 | 6 |
| | Total | 60 | 9 | 69 | 28 | 3 | 3 | 2 | 36 |
| B-72 albinos strain | Proestrus | 12 | 2 | 14 | 5 | 0 | 0 | 0 | 5 |
| | Oestrus | 11 | 1 | 12 | 5 | 0 | 2 | 0 | 7 |
| | Metoestrus | 2 | 1 | 3 | 2 | 0 | 0 | 0 | 2 |
| | Dioestrus | 3 | 0 | 3 | 4 | 0 | 0 | 0 | 4 |
| | Late Dioestrus | 5 | 0 | 5 | 2 | 0 | 0 | 0 | 2 |
| | Total | 33 | 4 | 37 | 18 | 0 | 2 | 0 | 20 |

mentioned are referable rather clearly to Text-figs. 3–4.

Some mention may be made on the seasonal variation in number of normal and abnormal ova. SIGEMORO (1944) studied the seasonal variation in number of abberant follicles from a series of sixty mature rats. The data presented by him indicated that the degenerating follicles in the ovaries represent no significant variation by season through the year. MAKINO, SIGEMORO & KOBAYASHI (1943) have studied seasonal variation of the normal follicles in female Norway rats, and examined the number of mature ova in ovaries through a year, reporting that the maximum number was found in the specimens secured in January and Febrary. The data presented in this study are insufficient to make any conclusion, since the present study




N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 4. Average number of normal, abnormal mature follicles and polyovular follicles in four mouse strains at different phases of the oestrous cycle.

was carried out from late summer to late autumn.

Part IV. Some histochemical observations on normal and abnormal ova in mouse ovaries

A considerable amount of literature has been published pertaining to the histochemical studies of glycogen, and nucleic acids in mammalian ova by many authors. TOGARI (1927), BRANDENBURG (1938) and HARTER (1948) have dealt with the

cytochemical demonstration of glycogen in rodent ovaries. They stated that the normal and atretic ova generally contained a large amount of glycogen. ISHIDA (1953, 1954) studied glycogen in the ovaries of several mammals, stated that normal ova observed in the secondary and Graafian follicles showed a extreme difference in glycogen content. Especially, rat and hamster ova contained glycogen in a large amount. He has found that atretic ova of rats were different remarkably in glycogen content; some ova contained no glycogen, but some others contained in a small or large amount.

VINCENT & DORNFELD (1948), JONES-SEATON (1950) and ISHIDA (1953) studied the contents of nucleic acids (RNA) in rodents eggs. According to them, a small amount of RNA was observed in different stages of the normal oestrous cycle of rats. ISHIDA (1953) studied RNA contents in ovaries of several mammals such as rats, hamsters, guinea-pigs, rabbits, goats, sheeps and cattles, and found no remarkable difference among them.

In the present study, the author has undertaken the study on the localization of glycogen and RNA in normal and abnormal ova in the ovaries of mature mice derived from Swiss albinos and D-240 albinos, 70 to 120 days in age. The results are as shown in the following. The work was carried out in 1960 and the results were partly reported.

(1) Glycogen content in ova

Use was made of four individuals of S-strain mice at four oestrous stages and two individuals of D-strain mice at the proestrus and oestrus stages. With the use of the PAS method (Periodic acid-Schiff method), the glycogen was demonstrated as red-purple granules. Seventy to eighty percent of normal ova from the primary follicles having one or more cell layers with no antrum were colored moderately red-purple in the surface (Fig.74). A few ova at the oestrus stage showed no existence of glycogen. A large amount of glycogen was observed in the majority of secondary normal ova in the follicles with a prominent antrum (Fig. 75), while a few others (10%) contained no glycogen (Fig. 76).

The atretic ova of large size proceeding beyond the maturation course contained glycogen without excetion (Figs. 77–78).

ISHIDA (1952) studied the localization of glycogen in the primary follicles in the ovaries of normal mature and immature rats, and reported that normal ova in primary follicles contained no glycogen, while secondary follicles were found to contain a large amount. TOGARI (1927), BRANDENBURG (1938), HARTER (1948) and some others, reported that all ova in secondary and Graafian follicles contained glycogen. ISHIDA (1954) studied glycogen contents in atretic ova in rats and hamsters undergoing fragmentation or cleavage during atresia, and reported that a large amount of glycogen was observed in atretic ova. But, in some other animals such as cattles, sheeps, pigs, goats, rabbits and guinea-pigs only a little or none of glycogen was found, except a large amount of glycoprotein.

The data obtained from this experiment indicated that the primary and second-

ary normal ova in mature mouse ovaries contained a varying amount of glycogen, in a few individuals the ova contained no glycogen, and that the atretic ova of larger size contained always glycogen.

The results of investigations have shown that there is no difference in content of glycogen by mouse strain, and further that there is also no remarkable difference in content of glycogen by different stages of the oestrous cycle of mice.

(2) RNA content in ova

RNA contents were studied in four individuals of S-strain mice at four stages in the oestrous cycle, with the use of a thionin staining method. Normal ova observed in both primary and secondary follicles showed a weak reaction to the test for RNA. This seems to indicate that follicles contain a small amount of RNA. A few atretic ova proceeding beyond the maturation course, 8 percent showed a reaction indicating the presence of a small amount of RNA.

JONES-SEATON (1950) reported that rat ova at every stage contained a small amount of RNA, and that with the development of the ova RNA increased in the cytoplasm. ISHIDA (1954) informed that normal follicles at every stage of the oestrous and atretic follicles at the early stage usually contained a small amount of RNA and that RNA showed no increase during the course of development of the ova.

The results of the present experiment are in agreement with those shown by ISHIDA (1954): RNA showed no remarkable increase during the development of ova.

## (IV) DISCUSSION

The present investigations have dealt with the morphology of the atretic follicles found in mouse ovaries, particularly on cytological features of abnormal mature ova proceeding beyond the maturation and polyovular ova found in the Graafian follicles of mature ovaries. The papers relating to atretic follicles have been published by many authors. ASAMI (1920) in the rabbit, ENGLE (1927) in the mouse, LANE (1938) in the immature rat, PLISKE (1940) in the ground squirrel and SIGEMORO (1947) in the Norway rat reported the morphology and structure of atretic ova occurring in the primary and secondary follicles. It was recoginized that a great many ova are produced in the mammalian ovary during the oestrous cycle, but the majority of them undergo degeneration, only a few attaining maturation course, and developing into functional ova. These authors did not inquire into the nature and behavior of atretic follicles in detail, and many questions have remained not answered on the meaning of their occurrence. The present author has undertook cytological observations from both morphological and cytochemical standpoints on the nature of atretic follicles, with the use of mouse ovaries derived from mice of different strains at 70 to 120 days of age. It was observed that the atretic follicles of larger size which seem to advance beyond the maturation course are very abundant in number in normal mature ovaries.

There were found several atretic follicles of larger size in which the ovum

showed the second polar body after the completion of the second maturation division, though the antrum folliculi has not been formed (Fig. 42). YAMANE (1930, 1935a & b, 1937) reported that insemination is an important anticident for the completion of the second division. The above abnormal examples showed the second polar body without insemination of spermatozoa. To the author's view, the second maturation division may proceed without insemination in the ovary under certain abnormal conditions. On the other hand, ASAMI (1929), BRANCA (1925), EREUD & VEDDER (1938) and PLISKE (1949), emphasized that the degeneration of abnormal follicles may cause by the disintegration of granulosa cells. It is probable from the author's results that the atretic ova may be produced by the influence of the hormonal secretion. Especially, the occurrence of aberrant follicles of large size which advance to the maturation course may justify the above view involving the influence of sex stimulating hormones.

Morphologically, the follicles are divided into two general types. Type 1 refers to the small primary follicles with several rows of granulosa cells, but having no antrum folliculi. Type 2 denotes the follicles which advance to the maturation course and have an prominent antrum in each. The sex stimulating hormone application is effective to only the largest follicles belonging to type 2.

The most important hormones produced by the mammlian ovary are those known as female hormones. Probably, the follicle stimulating hormones of the anterior pituitary seem to induce rapid growth of a Graafian follicle; the growth may be partly achieved by mulitiplication of follicles cells and partly by the hormonal secretion into the antrum of the follicular fluid. The pituitary hormones stimulate the gonad in two ways. First, they cause to secrete their own hormones (oestrogen in the ovary), and second they work together with these hormone to induce the maturation and escape of the germ cells. It is possible to consider that the gonadotrophic hormones of the anterior pituitary concerned for the most part with the separation of these hormones into follicles stimulating (F.S.H.) and ovulating or luteinizing (L.H.) fractions. This also applies to the other gonadotrophic hormones such as the luteinizing hormone obtained from the urine of pregnant woman (P.U.) and the follicles stimulating hormone which is found in the blood serum of the mare between the 45th and 100th day of pregnancy (P.M.S.).

Next, the author's study has been extented to analyse the effect of hormones on the atretic follicles with particular concern to the reduction of those follicles, with the application of anterior pituitary sex gland stimulating hormone (A.P.H.), the sex gland stimulating hormones of placenta (Cholionic gonadotrophin) and pregnant mare serum of horses (P.M.S.). The effect of sex stimulating hormones on abnormal mature ova of mice is rather remarkable. Especially, in the P.M.S. treated mice the remarkable decrease occurred in number of abnormal mature ova. Further, the decrease in number of abnormal ova showing immature condition of granulosa cells is remarkable. On the other hand, many atretic primary follicles were observed in the ovaries under the condition of hormone treatment. This evidence indicates that the hormones are not effective on the atretic primary follicles.

However, the present investigations have failed to produce any conclusive results on the question that the administration of any hormone would affect for the reduction of the atretic mature follicles during the normal oestrous cycle. In general, there are individual differences in mice which received injection of different hormone preparations.

LANE (1938) dealt with aberrant ovarian follicles in immature rats, and reported that the incidence of atypical follicles was not materially altered under the experimental condition in animals treated with various hormone preparations. PINCUS & ENZUMANN (1947) studied the growth, maturation and atresia of ovarian eggs of rabbits. According to them, the percentage of atresia among the young oocyte is low (10%); the larger follicles belonging to the secondary type showing about 60 percent are atretic at all stages of the reproductive cycle; they stated that the hormonal effect are on the secondary atretic follicles in the rabbit.

Numerous workers have shown how, by injection of hormones of various kinds, the number of ova may be considerably increased. Microscopical observations of artificially induced polyovulation through the injection of various hormones were reported by PINCUS (1940), PARKES (1943) in the rabbit, RUNNER & PALM (1953) in the immature mouse, GATES (1956) & GREEN (1956) in the mouse, OKIGAKI (1958) in the mature rat and SATO (1959) in the mature and immature mouse, and some others. On the other hand, ROBINSON (1949), HUNTER, ADAM & ROWSON (1955), RASIDA & LAMOND (1956) and others reported polyovulation in mature and immature domestic animals, under the administration of hormones such as anterior pituitary sex stimulating hormones or sex gland stimulating hormones or placenta or pregnant mare serum of horses or oestrogen. Their experiments have provided no evidence for effective results on polyovulation when single injection of P.M.S. hormone was made. But, most of the above results have offered no important analytical evidence for the mechanism of polyovulation under the influence of sex stimulating hormones.

Based on the data obtained from the present observations, the following assumption is possible that abnormal mature ova which may be failed to ovulate in untreat state may undergo ovulation under the effect of sex stimulating hormones, while such hormones may exert no effects on the primary atretic follicles having one or more cell layers with no antrum.

The widespread occurrence of the polyovular follicle has been reported in many kinds of mammals by HARTMAN (1926), ENGLE (1927), LANE (1938), DEDERER (1934), TAKEWAKI (1937), LANE (1938), SIGEMORO & MAKINO (1946), and SIGEMORO (1947). According to them, the aberrant forms of polyovular follicles are generally more common in immature and in older animals than in mature ones. But, SIGEMORO (1947) dealt with morphological observations of abnormal follicles in sixty mature wild rats, and reported that the polyovular follicles were abundant in number in the mature ovaries, being 275 in total.

According to the results of the present study which was based on mice at 70 to 120 days of age, polyovular follicles were few in number in occurrence; 80 poly-

ovular follicles were found to occur in 60 ovaries. Of them, almost 78 percent were biovular, in accordance with the results by the other authors. The author observed the ovaries of immature mice at 10 to 20 days of age, and found that the polyovular follicles are rather rare in occurrence; in 20 ovaries from a series of ten normal mice, 13 polyovular follicles and 1 binuclear ova were found in total. The data from the present observations indicated that the polyovular follicles were less common in mature and immature mice than in other mammals in general.

The present author is of opinion that the polyovular follicles may be formed through one of the following three processes: 1) polyovular follicles presented by concresence of previously separated follicles as shown in Fig. 63, 2) polyovular ova presented by abnormal nuclear division as shown in Fig. 62, and 3) polyovular follicles arised by separation or fragmentation of the original ovum as shown in Fig. 53. Type 1 and 2 seem to be common in occurrence. They may be in process of degeneration, though there are a few examples which possibly suggest their normal ovulation course. Type 3 may be doomed to atrophy in the ovary.

Concerning the origin of the polyovular follicles, HARTMAN (1926) enumerated three possible ways of formation; 1) by division of polyovulation ova, 2) by concrescence of previously separated follicles, and 3) by persistent union of ova in the egg tube.

KENT (1959) reported that occurrence of polyovular follicles and polynuclear ova were rather common in immature animals. He assumed that polyovular follicles may be produced due to alleviation of a deficient estrogen condition in the young golden-hamster. He showed that polyovular follicles and polynuclear ova was reduced 16 to 18 percent of the control value through daily injections of estradiol monobenzoate in seame oil.

The results of the present study indicated that polyovular follicles were slightly reduced in number in P.M.S. treated mice than in the untreated mice. But, no conclusive statement was made whether or not the administration of the sex stimulating hormones would also reduce the number of polyovular ova.

Further, evidence has been presented by the author that there is no remarkable difference in number of normal and abnormal mature ova and polyovular follicles among the following four strains of mice; Swiss albinos, D-240 albinos, dd/Ma albinos and B-72 albinos. In the four strains of mice, abnormal ova which advance towards the maturation course were very abundant in number in mature ovaries. Also, polyovular ova were rather rare in occurrence.

The oestrous cycle was observed in four mouse strains, and the following results were obtained that there is a variation in their cycle. It is noticeable that a few S-strain and D-strain mice showed some abnormality in sexual cycle. They showed an abnormally long dioestrus stage.

Some histochemical studies were undertaken on the normal and abnormal ova as regard to the charge in occurrence and localization of glycogen and nucleic acids. Recently, ISHIDA (1953) reported that the atretic ova of rat ovaries began atrophy through the following three types of degeneration process, (1) the nucleus of the

ovum showed the piknosis and its cytoplasm shrunk without fragmentation or cleavage, (2) the ovum underwent symmetrical cell division until about 2 to 8 cell stages, and then their cytoplasm became irregular and finally disintegrated, and (3) the cytoplasm of the ovum showed irregular cell division by which irregular cell masses were formed. He stated that the three degeneration types occurred in parallel condition to the appearance of glycogen in ovaries. The ova of rats and hamsters undergoing fragmentation or cleavavge during atresia were generally found to contain a large amount of glycogen, while those of cattles, sheeps, pigs, goats, rabbits and guinea-pigs undergoing shrinkage during atresia contained a little or none of glycogen. The present study with mice has resulted in that atresia ova have been found to contain at various stages glycogen without exception. Some atretic ova proceeding the maturation course contained a small amount of glycogen, though sometimes a large amount of glycogen was found to occur. In the present study, no positive evidence has so far been obtained to show that there is any relationship between the charge in amount of glycogen and the formation of atretic ova.

## (V) SUMMARY

The data obtained in the present investigations were described being separated into four parts.

Part I is devoted to the cytological studies of normal, abnormal mature ova and polyovular follicles in mature mice, *Mus musculus* (Swiss albinos) during the normal sexual cycle. It was found that the atretic follicles of large size in which the ova were in a course of maturation were very abundant in number in ovaries of mature mice. It seems probable that the course of degeneration takes place in both egg body and granulosa cells, and that the results affect the hormone secretion in the mature ovary. The polyovular follicles were found to be rather rare in occurrence both mature and immature mice.

In Part II is described an cytological effect of sex stimulating hormones on large abnormal follicles and ova in *Mus musculus* (Swiss albinos). The experiments were carried out by two ways. The first dealt with the effect of the mixture of anterior pituitary sex gland stimulating hormone and sex gland stimulating hormones from placenta upon the fate of abnormal follicles. The second was to examine the effect of the mixed hormones as above and the pregnant mare serum of horse upon abnormal follicles.

On the basis of the results of the present observations, the conclusion is possible that the reduction in number of abnormal mature ova may be explicable by the effect of sex stimulating hormones. Also, the evidence presented in the two experiments has indicated that abnormal mature ova showed a remarkable decrease in number in pregnant mare serum treated mice. Especially, the hormone adminstration probably affects only the follicles of larger size. Further, the number of abnormal ova showing an immature condition of granulosa cells is remarkable. From the results of the present study, it is presumed that abnormal mature ova which

failed to ovulate may undergo ovulation under the effect of the sex stimulating hormones.

Part III describes the results of the cytological investigation of some comparative features of normal, abnormal mature ova and polyovular follicles in mature ovaries of the following mouse strains: Swiss albinos, D-240 albinos, B-72 albinos and dd/Ma albinos. There is no remarkable difference in number of normal, abnormal mature ova and polyovular follicles by mouse strain.

Part IV gives accounts of the histochemical observations of normal and abnormal ova in mature ovaries of mice studied at four stages of the oestrous cycle. The occurrence and localization of glycogen and RNA were the subjects mainly dealts with in ovaries of *Mus musculs* (Swiss albinos and D-240 albinos). The data derived from this experiment indiacted that the primary and secondary normal ova in mature ovaries contained a varying amount of glycogen, that in a few individuals the ova contained no glycogen, and further that the atretic ova of large size contained always glycogen. Normal ova observed in both primary and secondary follicles showed a weak reaction to the test for RNA. This seems to suggest that the follicles contain a small amount of RNA. A few atretic ova preceding beyond the maturation course showed a reaction indicating the presence of a small amount of RNA.

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## (VII) EXPLANATION OF PLATES 1–13

### Plate 1.

Figs. 1–6. Photomicrographs of Graafian follicles showing the normal course of maturation in the mature mice.

Fig. 1. Graafian follicles containing a full grown egg nearing maturity.

Figs. 2–4. Ova showing the disintegration of nucleoli.

Figs. 5–6. Ova nearing the first maturation division after the disappearance of the nuclear membrane. Visible well-defined bivalent chromosomes are found in the nucleus.

### Plate 2.

Figs. 7–12. Ova showing the normal first maturation division at the side and polar view. Graafian follicles are found to have a prominent antrum.

### Plate 3.

Figs. 13–18. Ova showing the normal first and second maturation division.

Figs. 13–14. Side view of the first division at metaphase in the egg.

Figs. 15–16. Ova showing the second polar spindle at metaphase, accompanied by the first polar body.
Figs. 17–18. Ova just before rupture. The maturation process in the ova stops at the metaphase stage of the second division.

## Plate 4.

Figs. 19–24. Abnormal Graafian follicles with a few antra showing the maturation process.
Figs. 19–20. Abnormal mature ova nearing first maturation division.
Figs. 21–24. Abnormal ova with a few antra showing the first division.

## Plate 5.

Figs. 25–30. Abnormal Graafian follicles showing the first maturation division, the antrum has not been fully formed.

## Plate 6.

Figs. 31–36. Abnormal ova with a few antra showing the first maturation spindle. The atretic follicles are in the process of degeneration and the disintegration takes place in the granulosa cells.

## Plate 7.

Figs. 37–42. Abnormal ova showing the second maturation division.
Figs. 37–41. Ova showing the second polar spindles at metaphase accompanied by the first polar body in the follicles with a few antra.
Fig. 42. Abnormal ovum showing the first and second polar bodies.

## Plate 8.

Figs. 43–48. Ova showing the abnormal maturation division with a prominent antrum.
Figs. 43–44. Chromosome abnormalities observed in mature ova.
Figs. 45–46. Abnormal ova showing piknosis of chromosomes at the first maturation division.
Figs. 47–48. Unshed ova are doomed to atrophy.

## Plate 9.

Figs. 49–54. Multinucleate ova and polyovular follicles.
Figs. 49–50. Ova showing the abnormal maturation division, the ova performed irregular cell division or cell fragmentation.
Figs. 51–52. Ova containing poly-nuclei.
Figs. 53–54. Polyovular follicles. The ova are doomed to atrophy.

## Plate 10.

Figs. 55–56. Polyovular follicles.
Fig. 57. Ovum showing the irregular two cell fragmentation.
Figs. 58–59. Abnormal ova showing vacuoles in the cytoplasm.
Fig. 60. Mature ovum showing the metaphase of the first division with tetra-polar spindles.

## Plate 11.

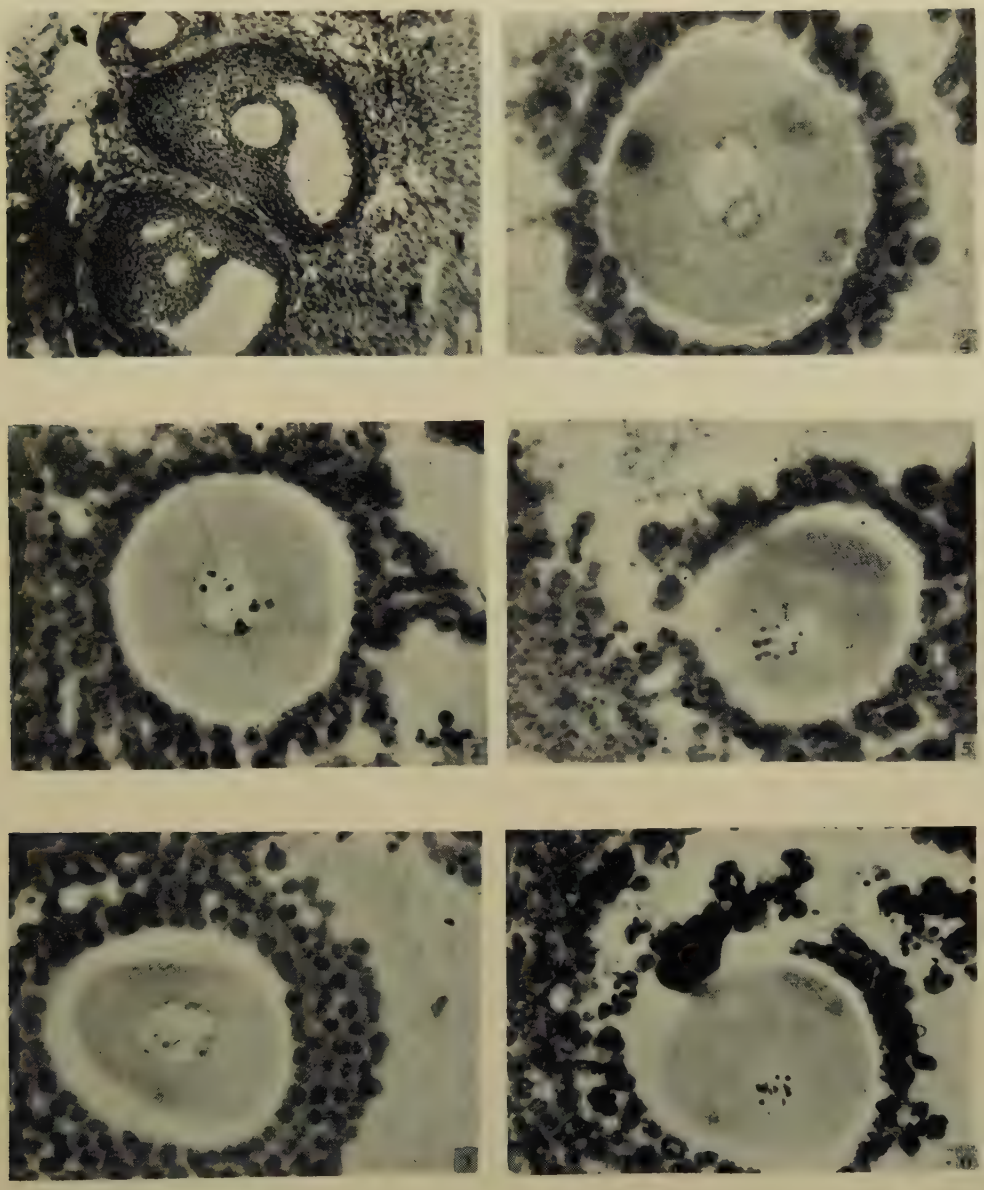
Fig. 61. Ovum showing tetra-polar spindle.
Fig. 62. Normal biovular follicle.
Figs. 63–65. Biovular follicles containing one ovum advancing towards the maturation, but other ovum is prior to maturation division.
Fig. 66. Biovular follicle arise by formation of the cell fragmentation.

## Plate 12.

Figs. 67–72. Ova of the immature mice.
Fig. 67. Many immature follicle contained ova in prior to maturation course in the ovaries.
Fig. 68. Bi-nucleate follicle.
Figs. 69–70. Unshed ova with no antrum showing the first maturation division.
Fig. 71. Degenerating ovum are doomed to atrophy.
Fig. 72. Abnormal polyovular follicles.

## Plate 13.

Fig. 73–78. Section of mature ovaries for the observation of glycogen content with the use of the PAS method.
Fig. 73. The ova contained a varying amount of glycogen granules.
Fig. 74. Primary follicles show a large or moderate amount of glycogen in the cytoplasm of ova.
Figs. 75–76. Secondary follicles show a small amount of glycogen in the cytoplasm of ova.
Figs. 77–78. Atretic ova show a large amount of glycogen granules.



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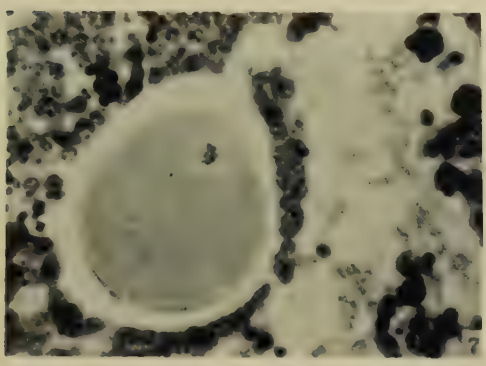


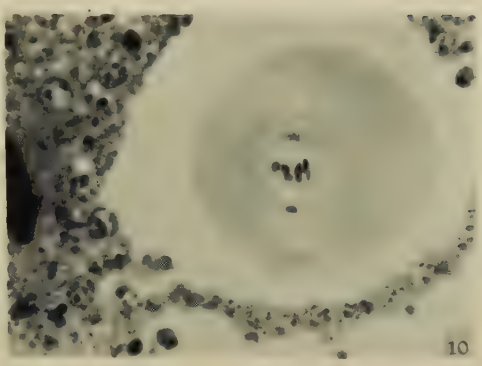


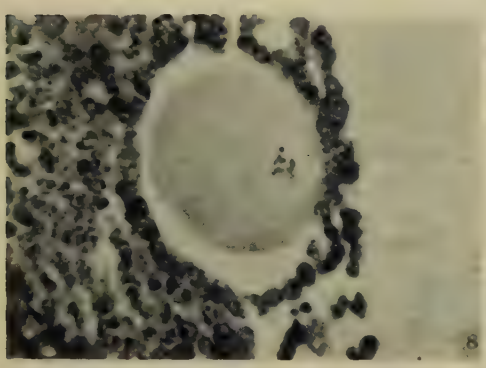


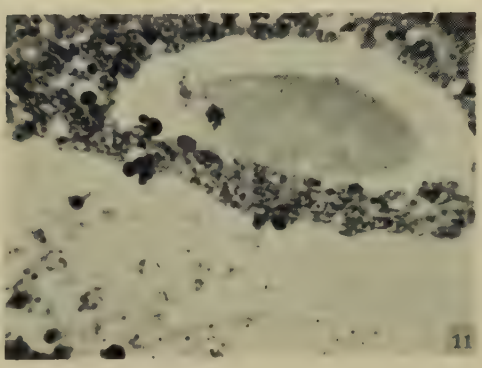


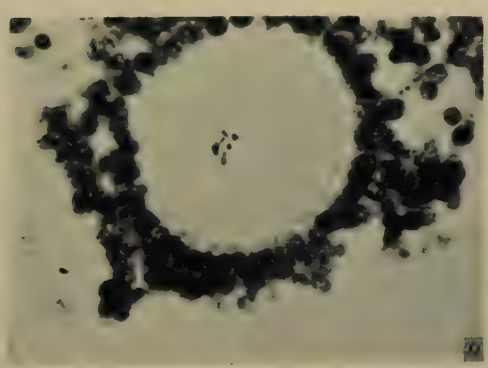


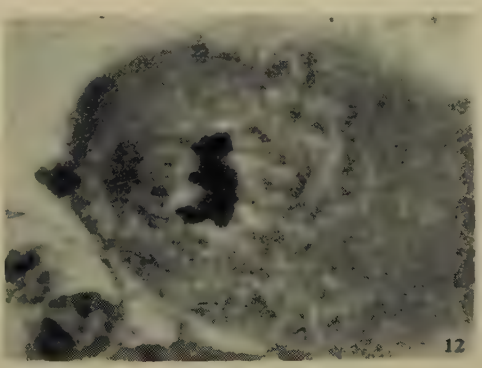


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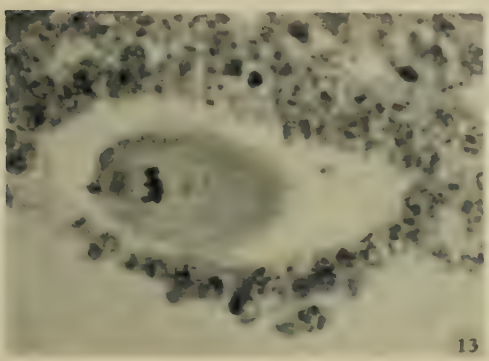


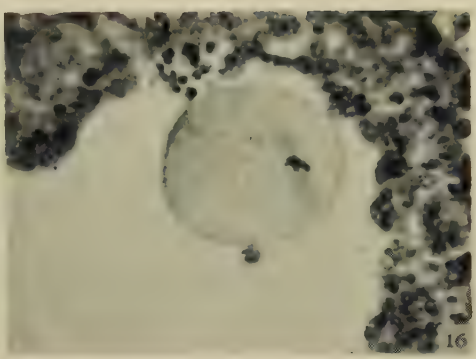


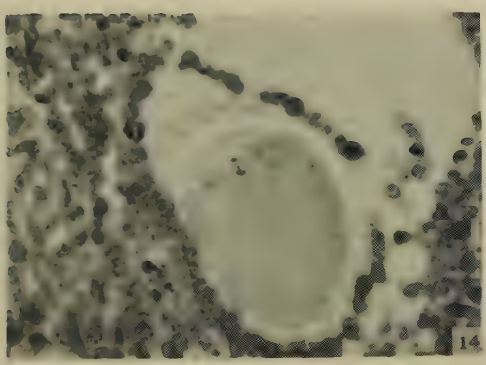


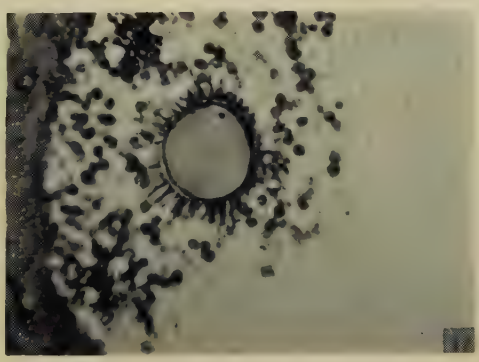

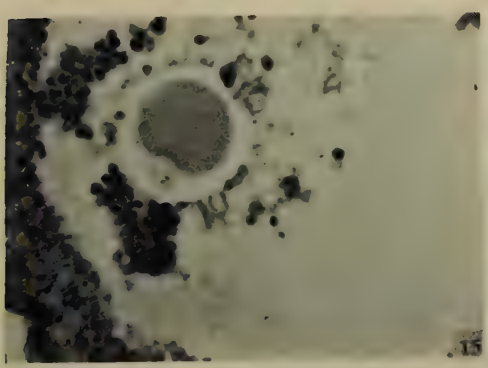


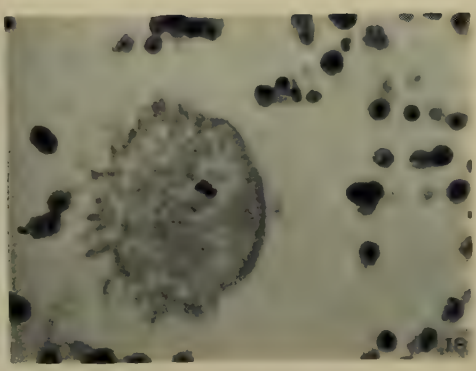


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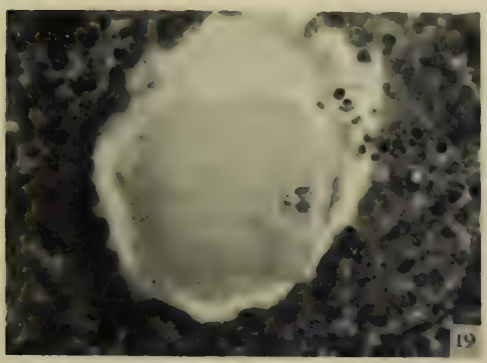


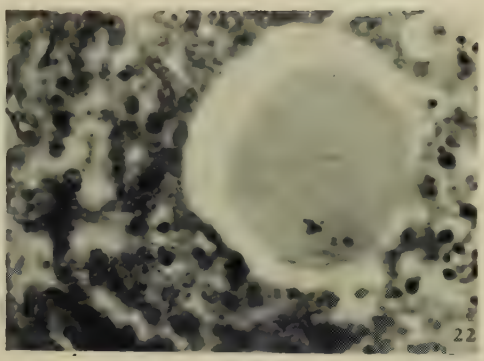


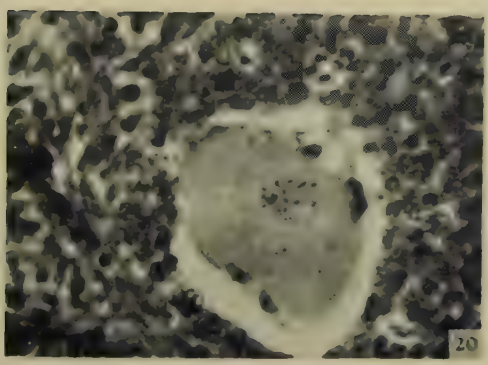


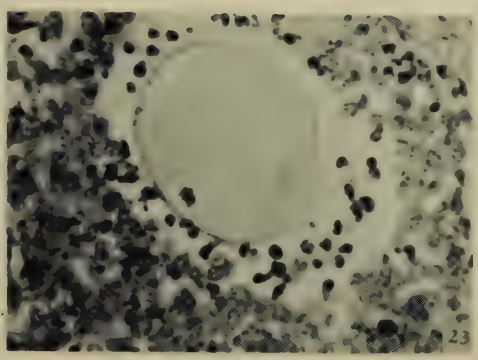


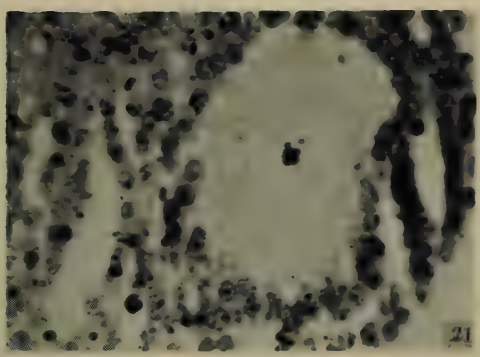


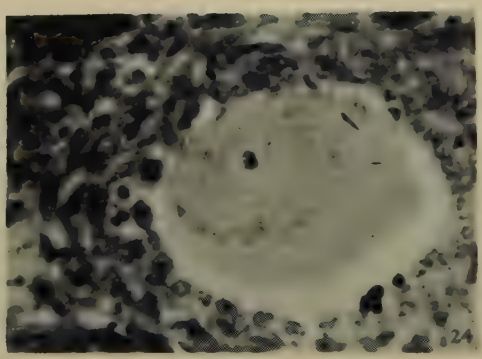


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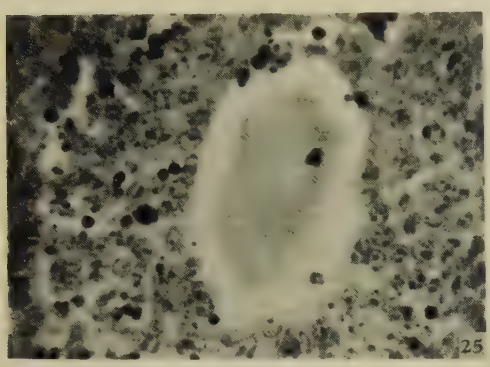


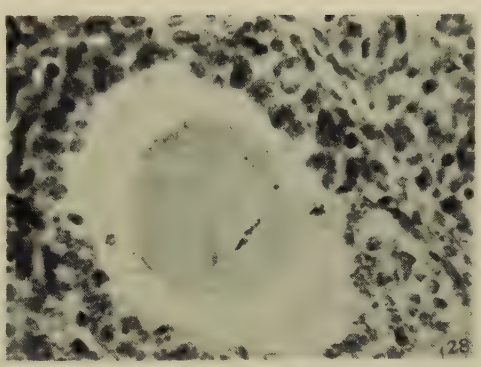


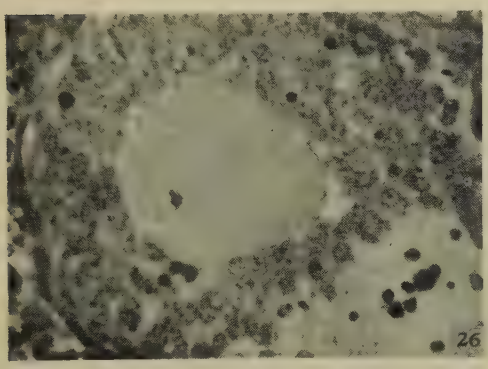


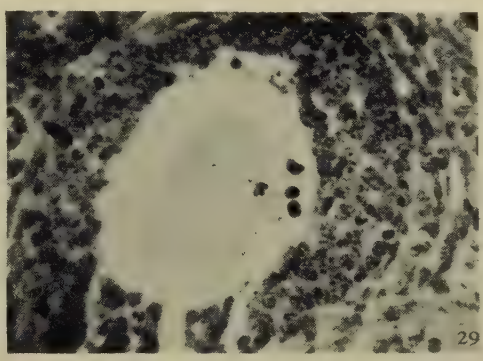


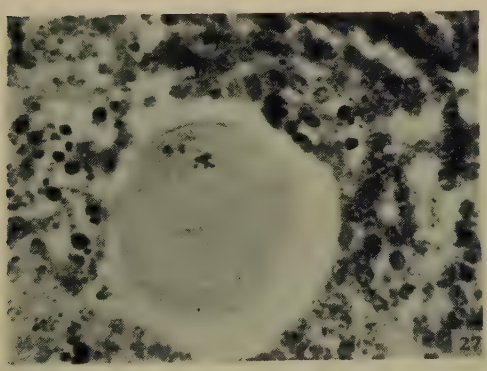


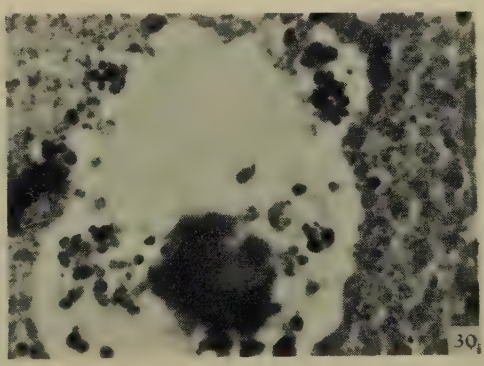


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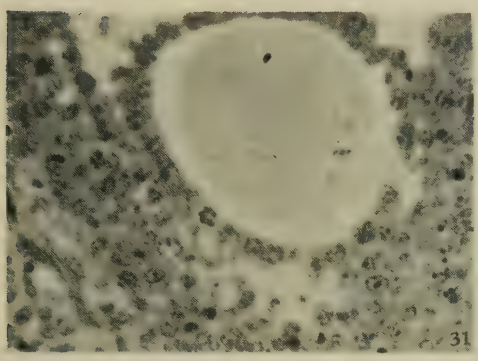


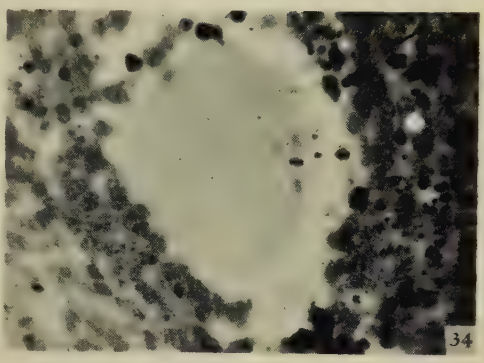


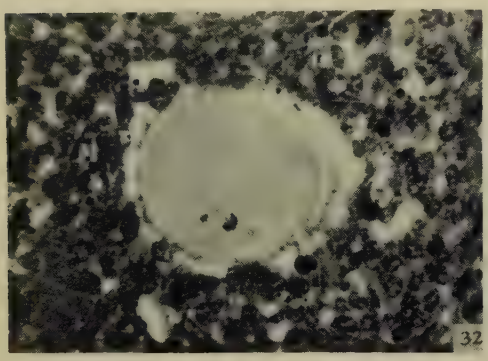


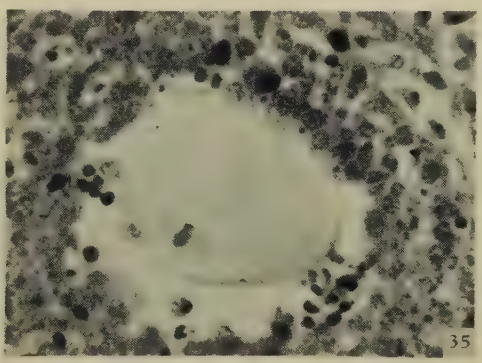


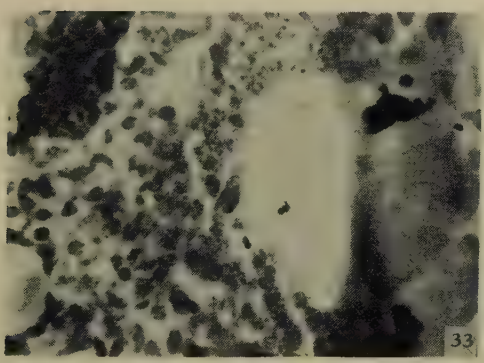


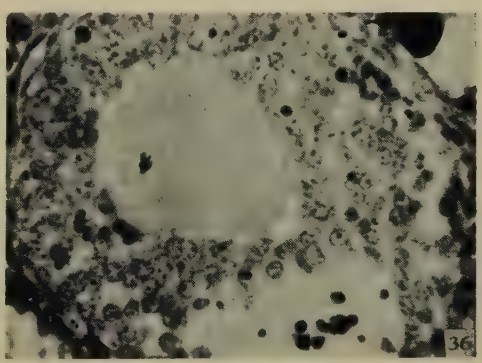


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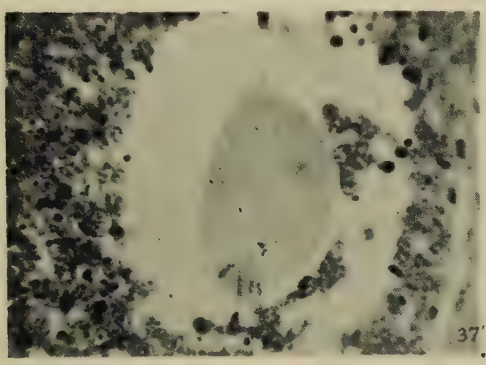


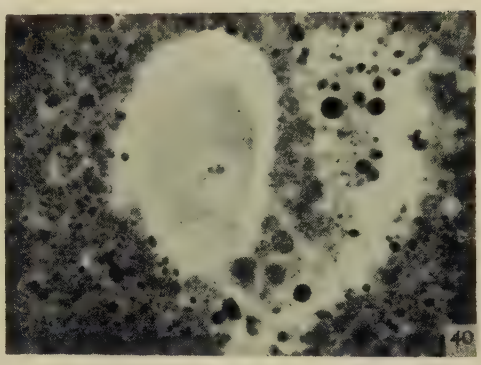


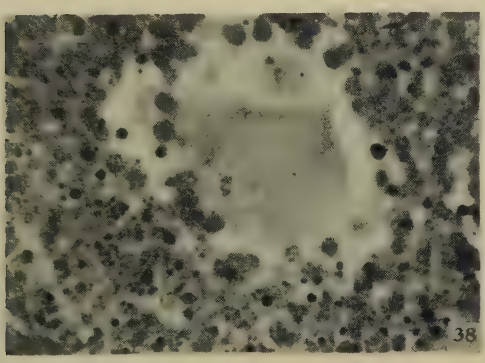


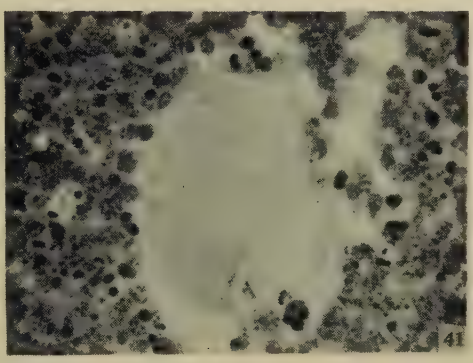


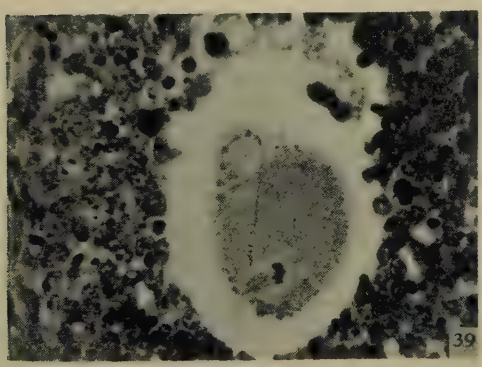


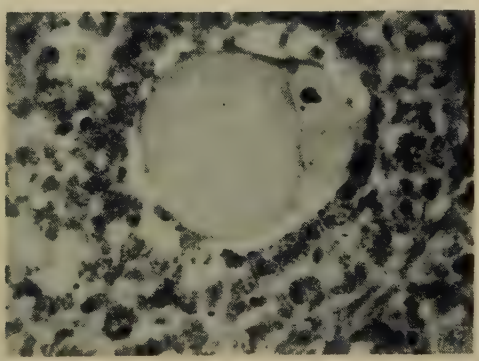

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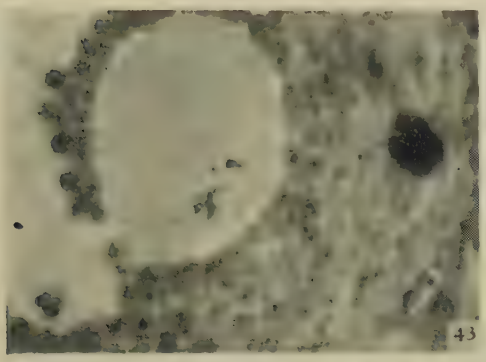


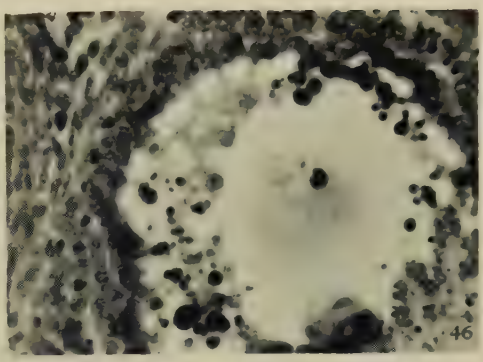


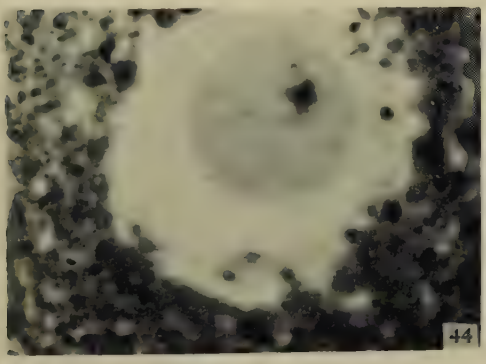


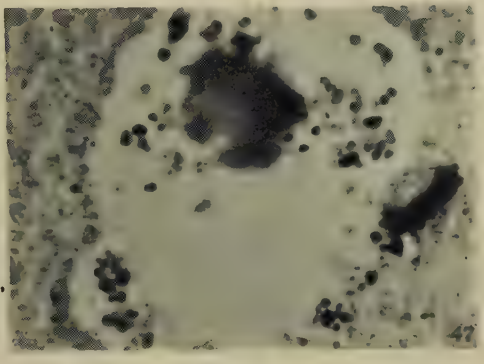


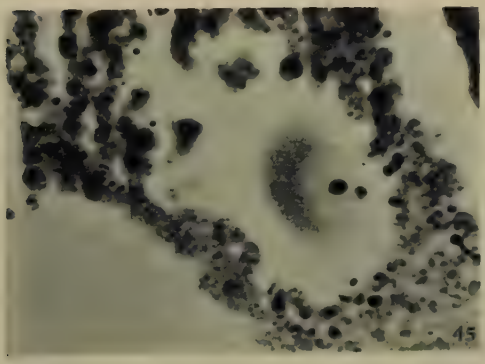


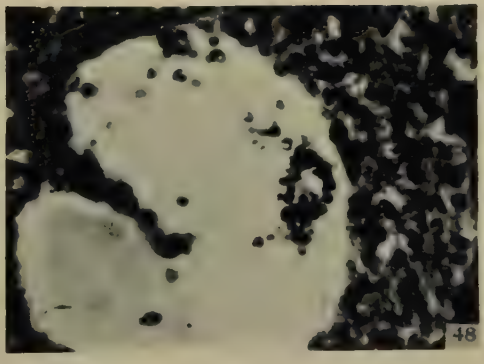


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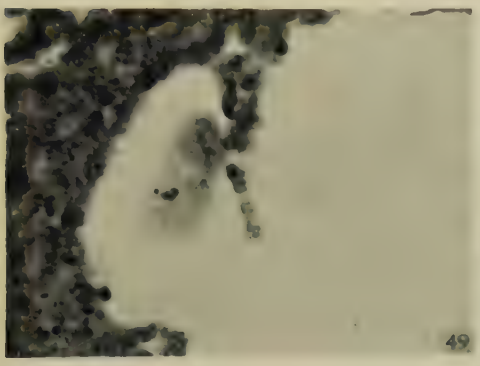


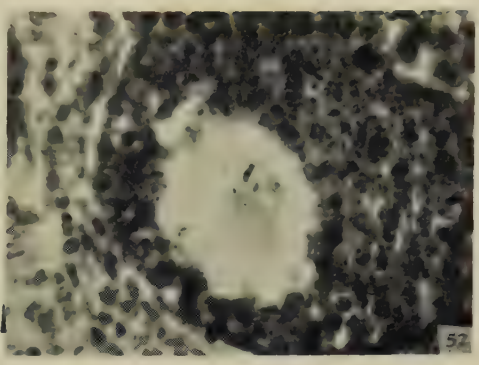


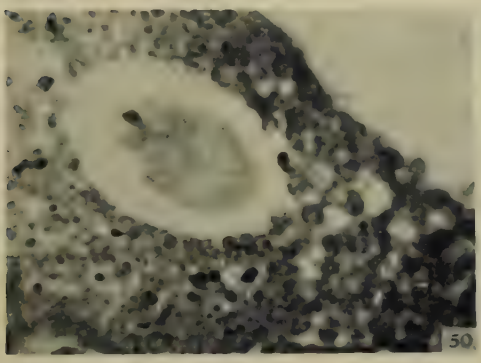


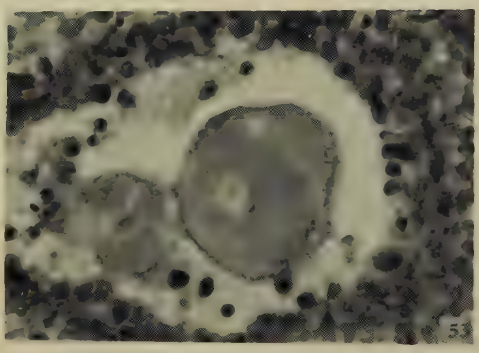


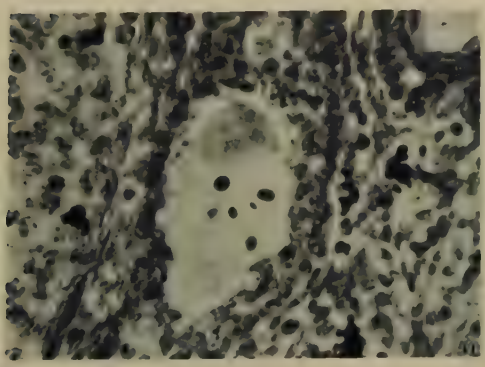

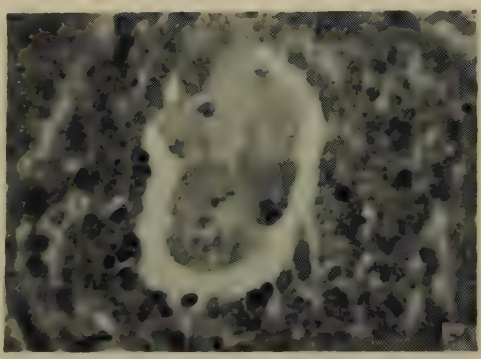

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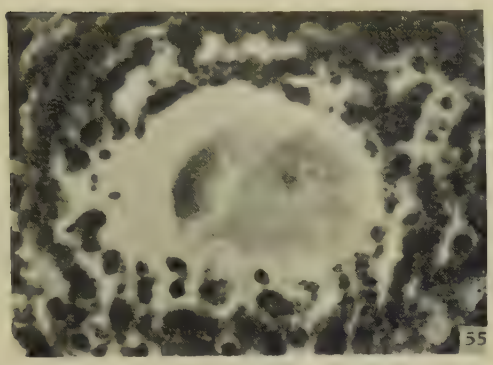


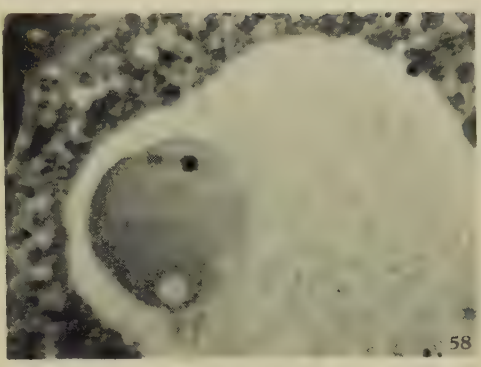


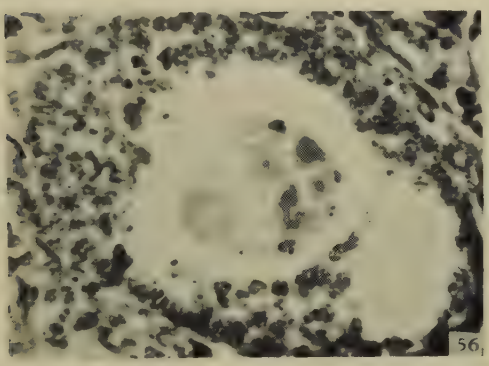


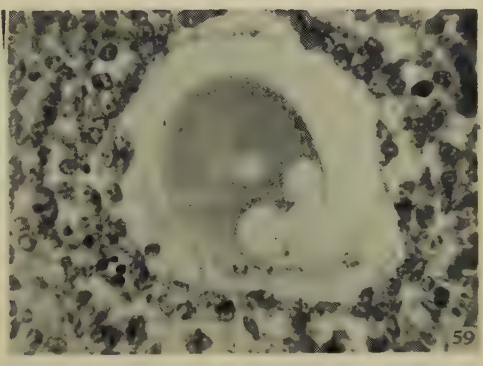


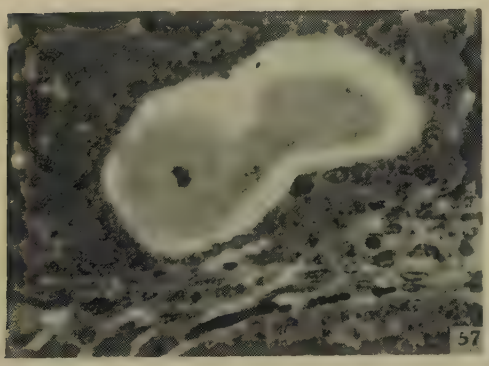


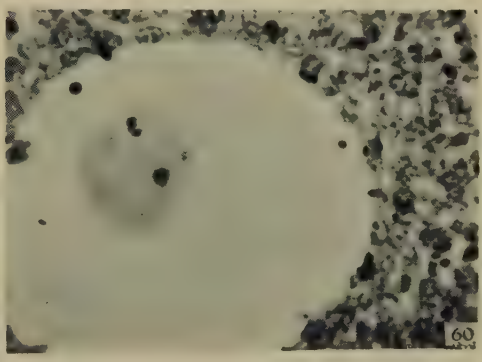


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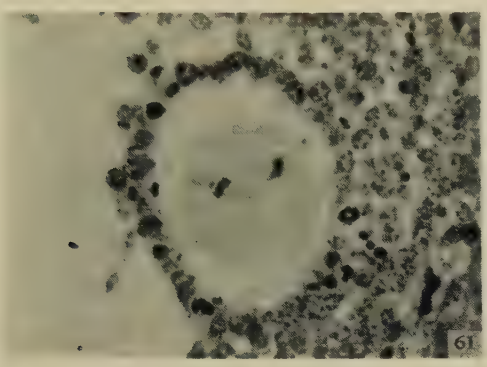


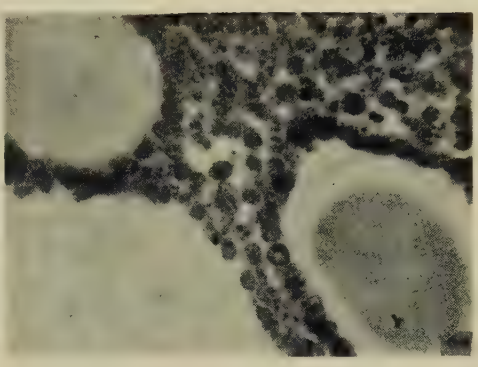

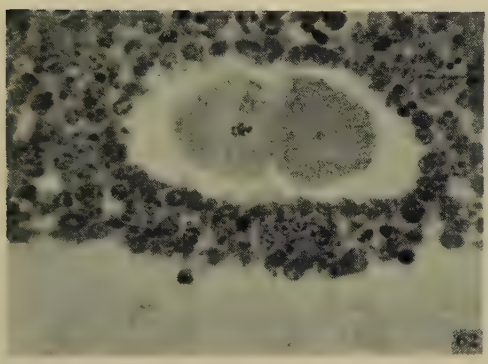

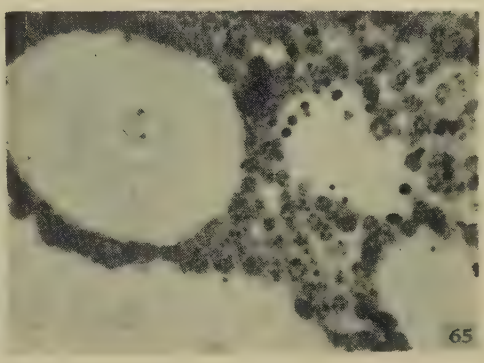


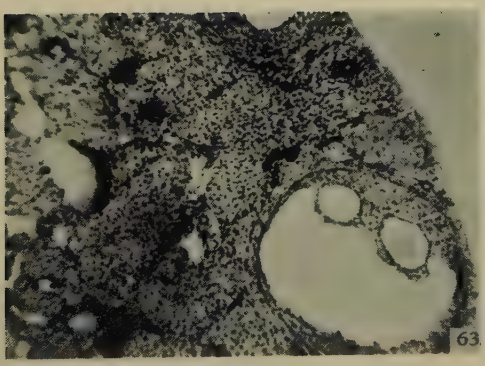


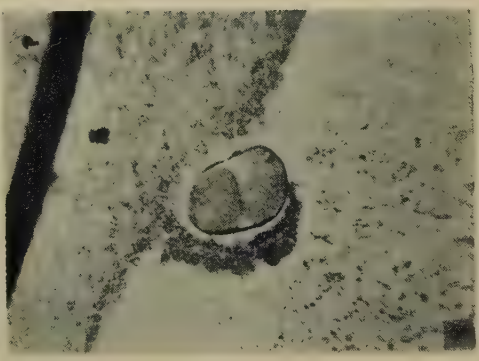

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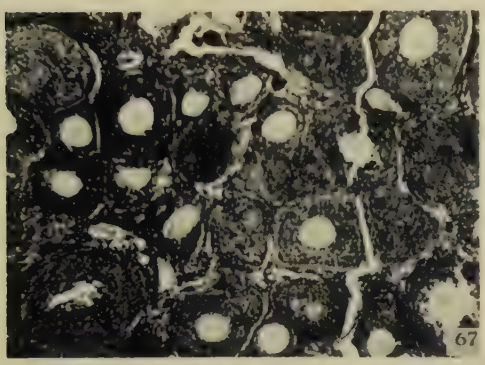


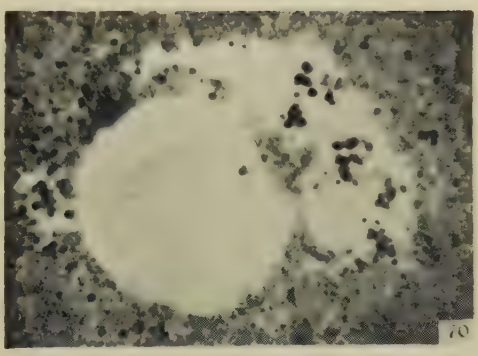


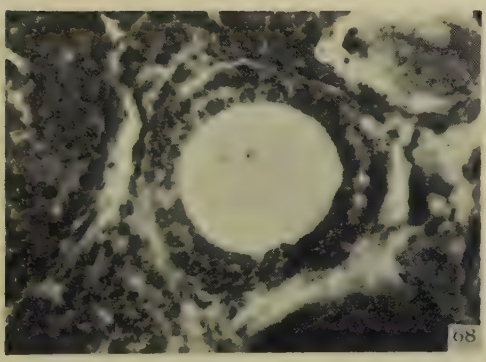


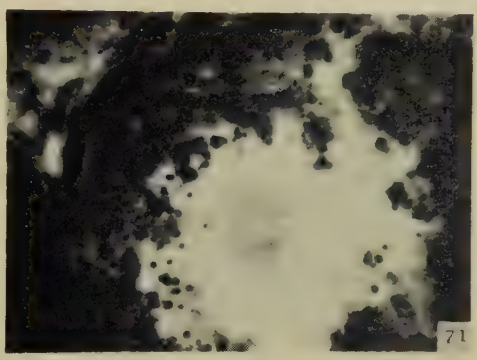


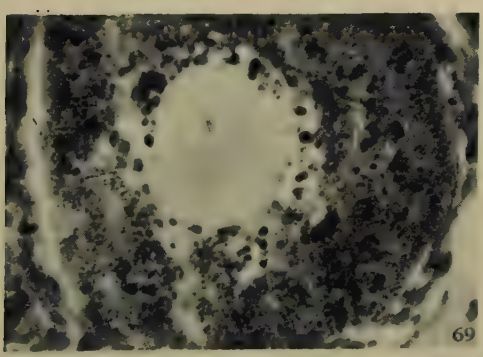


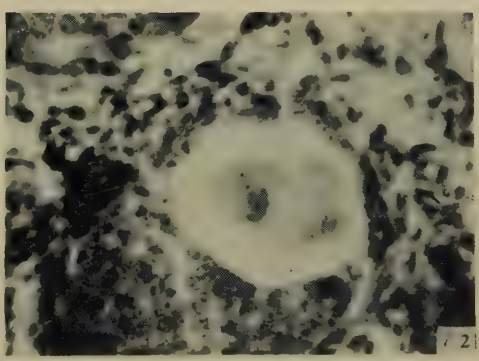


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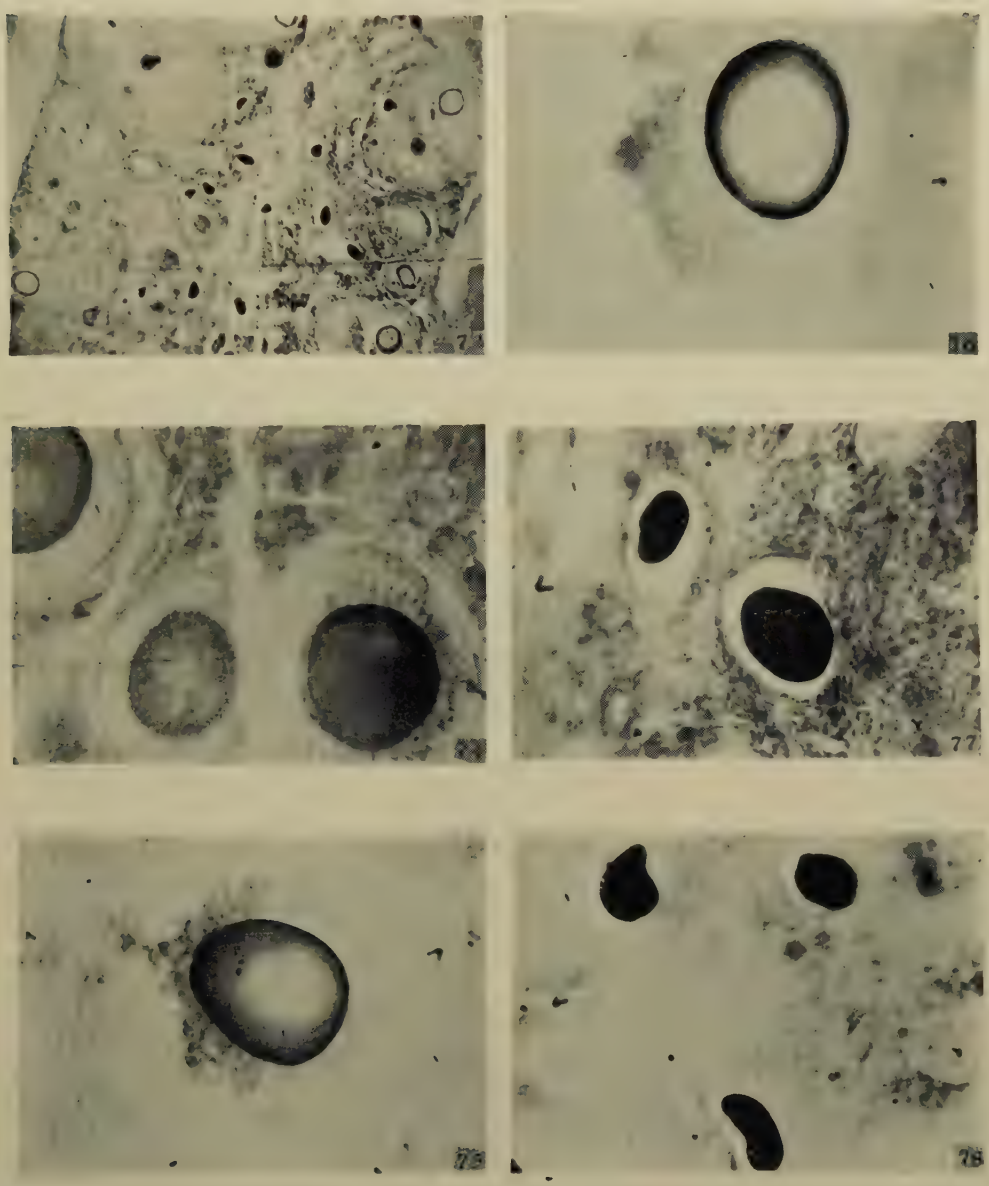


NAKAMURA : Abnormal Follicles of Mice

# Experimental Studies on the Artificial Insemination of Domestic Ducks with Special Reference to the Production of Mule-Ducks

Moriyuki WATANABE

*Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Plates 1–4, Text-figures 1–5, Tables 1–14)

## CONTENTS

I. INTRODUCTION ..... 440
II. COPULATORY ORGANS AND COPULATORY BEHAVIOR OF COMMON DUCKS ..... 441
1. Copulatory Organs ..... 441
2. Copulatory Behavior ..... 442
III. TECHNIQUES OF SEMEN COLLECTION ..... 442
1. Application of Massage Method ..... 443
2. Application of Electrical Stimulation ..... 445
Summary ..... 446
IV. CHARACTERISTICS OF SEMEN OF COMMON DUCKS ..... 447
1. Characteristics of Semen Collected by Massage Method ..... 447
2. Characteristics of Semen Collected by Electrical Stimulation ..... 448
Summary ..... 451
V. TECHNIQUES OF SEMEN INTRODUCTION ..... 451
1. Techniques of Semen Introduction by Palpation ..... 452
a. Experiments with the semen collected by massage ..... 452
b. Experiments with the semen collected by electrical stimulation ..... 454
2. Techniques of Semen Introduction by Employing a Speculum ..... 455
Summary ..... 456
VI. EFFECTS OF DILUTORS AND STORAGE ON FERTILITY OF DUCK SEMEN ..... 457
1. Effects of Dilution Rate ..... 457
2. Effects of Egg-yolk Citrate Buffers ..... 458
3. Storage and Fertility of Semen in Egg-yolk Buffer ..... 459
Summary ..... 460
VII. APPLICATION OF ARTIFICIAL INSEMINATION FOR MULE-DUCK PRODUCTION ..... 461
1. Copulatory Behavior of Muscovy Drake in Mating with Common Duck ..... 461
2. Collection of Semen from Muscovy Drake ..... 462
3. Characteristics of Muscovy Semen ..... 462
4. Insemination of Common Ducks with Muscovy Semen ..... 464
5. Hatchability, Pre- and Postnatal Development of Mule-Ducks ..... 465
Summary ..... 467
VIII. DISCUSSION ..... 467
1. Techniques of Semen Collection ..... 468
2. Techniques of Semen Introduction ..... 470
3. Characteristics of Semen of Common Ducks ..... 470

4. Effects of Dilution Rates of Semen of Common Ducks upon Fertility .....471
5. Effects of Chemical Composition of Dilutors upon Fertility of Semen of Common Ducks .....472
6. Mule-Duck Production by Applying Artificial Insemination .....473
IX. CONCLUSION .....475
REFERENCES .....476
EXPLANATION OF PLATES .....478

## I. INTRODUCTION

Duck raising in Japan is a relatively small industry. Its relative smallness, however, does not mean that its production is valueless. In fact, Kyoto and Osaka of Middle Japan are great consuming cities of duck meat. Also in many countries of Asia, the duck is mostly produced by a family industry, but its eggs and meat are very valuable resources of animal protein foods. The indispensability of duck meat accompanied with pork in Chinese dishes is wellknown, not to mention, that the world-famous Pekin duck was bred some centuries ago for this purpose. Beside the great domestic consumption, the duck egg processed in various ways as salted, powdered etc. is an important export of China. For the Filippino, the boild duck-egg, called balut, is a delicacy; balut is an egg in which the embryo has been allowed to develop usually up to the thirteenth or nineteenth day of incubation (BURGOS, 1934).

Duck raising, particularly in South-East Asia, is very closely connected with the rice cultivation. The majority of the inhabitants of this area are good duck-raisers keeping ducks around their living houses. Most of them purchase ducklings from hatcheries and rear them by providing only kitchen or garden refuse, sometimes with a small amount of paddy or rice-bran, while the greater part of feeds is sought by ducklings themselves in the nearby waters as ditches, brooks, pools, paddy-fields, etc. The total amount of eggs and meat thus produced is enormous; although the most part of it is consumed at home, hence statistics are not available.

In addition, it is a common custom among the inhabitants of South-East Asia to raise the hybrid bird between the Common duck (*Anas platyrhyncha var. domestica*) and the Muscovy drake (*Cairina moschata*). This hybrid bird is commonly known as mule-duck.[1] The mule- duck, although completely sterile in both sexes, has much merit as a meat producer, exhibiting a rapid growth, a strong constitution and good foraging qualities due to heterosis. In Taiwan, for example, the Common duck of penguin type is bred for egg production and called *tsai-ah* (菜鴨), and the Muscovy duck, named *fan-ah* (蕃鴨) is kept for the production of the mule-duck, which is known as *too-fan-ah* (土蕃鴨); the mule-duck is raised exclusively for meat purpose. The same method of duck keeping for the production of eggs and meat seems to be prevalent all over the areas of South-East Asia, not only among the Oversea-Chinese but also natives.

The domestic duck, i.e. the Common duck, is a polygamous bird, but its polygamous habit is not so extensive as the fowl. As a matter of fact, in China the

1) According to KURODA (1958) the mule-duck is known in binominal terms as *Anas hybrida*, *Anas maxima* and *Anas sterilis*.

breeder of Pekin-duck mates a drake with four ducks at most in order to obtain 80 percent of the resultant fertility of eggs (KUMASAKA, 1942). Also, the low fertility of intergeneric eggs in the mule-duck production is often reported. In view of such a low fertility of the Common duck as well as the Muscovy, it is considered that the application of artificial insemination to the duck breeding should be of great significance for the production of the largest possible number of progeny.

The artificial insemination, however, has not so far been applied to the duck breeding industry, perhaps for two reasons: Little understanding of the nature of this industry and the technical incompleteness of insemination.

From this standpoint, the present experiments were performed from 1953 to 1960 at the Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Hiroshima Prefecture. The research program was concerned primarily with improving the technique of artificial insemination for applying to the Common duck and secondly with promoting the fertility rate of hybrid eggs between the Muscovy drake and the Common duck by using the improved technique.

The author wishes to express his sincere thanks to Prof. emer. Dr. J. YAMANE, President of Hiroshima Agricultural College, formerly Dean of Faculty of Fisheries and Animal Husbandry, Hiroshima University, for suggesting this study as well as for constant guidance and encouragement during the course of the work.

## II. COPULATORY ORGANS AND COPULATORY BEHAVIOR OF COMMON DUCKS

Since the structure of copulatory organs and the copulatory behavior of the Common duck seem to be fundamental knowledge for the application of artificial insemination to this bird, some observations should be first taken in consideration.

### 1. Copulatory Organs

The drake, unlike the cock, has a long penis which is developed from the phallus in embryonal stages. According to HASHIMOTO (1939), the phallus begins to develop into an elongated penis when the duckling is 5 months old. In the grown bird its length attains 8–10 cm. Normally the penis is found involuted in a membranous fold in the ventral wall of the cloaca. On sexual excitement the penis evaginates itself spiralling two and a half or three times outside of the cloaca. Along the spiral of the penis a groove, *sulcus ejaculatorius,* passes from the urodeum of the cloaca to the apex of the penis. On erection of the penis, both ridges of the sulcus come in contact so closely with each other that it forms a canal-like passage, through which semen flows out at ejaculation. Since the ureters open into the urodeum, the urinary and reproductive ducts in ducks, unlike mammals, are completely separated, although the penis is developed. In the center of the penis a mucous canal runs longitudinaly, leading from its root to its apex. While the canal ends in blindness at the root of the penis, it opens at the apex of the penis and secrets mucus which lubricates the evagination of the penis and facilitates the copulatory act. Most peculiar fact is that the wall of this mucous canal serves as a sheath for the invagination of the penis upon diminishing of its erection. HASHIMOTO

(1939) found a great number of Herbst's corpuscle, an end organ of nerves peculiar to the bird, in the submucosa at the root of the penis, and suggested that this part should be most sensitive to sexual excitement of the drake.

In the female duck, the organ homologous to the penis of drake remains merely as rudimentary phallus, representing a slightly flattened protuberance throughout the life. Whereas the rectal opening is seen on the median wall of the cloaca, the vagina leads to the dorso-sinistral wall of the cloaca because of the unilateral development of the Müllerian ducts. The vagina measuring about 2 cm. in length leads from the uterus with a slight bend at its root.

Comparing the full length of the penis at erection with the depth of the cloaca (about 1.0 cm.) and the length of the vagina (about 2.0 cm.), it seems to be logical to deduce that at copulation the penis is inserted through the vaginal orifice as deep as the uterus. On this account, the spiralling of the penis appears to be very fitted for its penetration into the deviated vaginal orifice, leading through the utero-vaginal bend.

### 2. Copulatory Behavior

In natural breeding the Common duck (*Anas platyrhyncha var. domestica*) has a water-copulatory habit but it can mate on land also if confined in a pen or left out of doors devoid of water. Present observations were made in a yard without any other pool or puddle than a basin for drinking water.

Prior to copulation, the drake approaches a duck bending his neck wave-like near the ground, and then pecks repeatedly at the nape of duck with his bill until the duck carries her body downward. The duck's carriage being low down enough, the drake seizes firmly the duck's nape with his bill and then mounts on the duck. Upon riding on her back, the drake exerts massage-like motions with his toes upon the duck's ilio-femoral regions and excites her vent with the erected penis for 10 to 20 seconds. When the excited duck keeps her vent upward enough, the penis is inserted momentarily through the vaginal orifice to reject the semen. The drake retreats from the duck's back after ejaculation, flaps strongly, runs to a basin and cleans his feathers all over the body. The time reqired for a copulatory act from seizing the duck to the ejaculation usually does not exceed 20–30 seconds.

The duck receiving the drake's mounting bends her tail upward and rhythmically opens and shuts the vent, sometimes secreting a mucus. At the penetration of the penis the duck quacks in a under-tone and after being freed from the drake, the duck also runs to a basin in order to clean her body thoroughly as the drake does.

## III. TECHNIQUES OF SEMEN COLLECTION

Present experiments were undertaken with the two aims: firstly to find out if there is any room for improvement in both massage and electrical stimulation methods, and secondly to determine the relative advantages and disadvantages of the two.

Ducks used here were of a Japanese White Breed, so-called "Osaka-duck" which

had been bred by crossing the native duck with the Pekin duck. The name of "Osaka-duck" is originated from the city of Osaka in Middle Japan where a great amount of duck meat is yearly consumed. Unlike the penguin form of native ducks of South-East Asia, the "Osaka" shows a canoe-type in body form; it is an outstanding meat-producer with rapid growth. The average weights are: adult drake, 2.3 kg and adult duck, 1.8 kg.

During the course of the experiments, the drakes were strictly isolated in individual coops day and night; the ducks were ranged ashore at day time but each confined into its own trap-nest from evening on. They were supplied with an ordinary forage of breeder ration.

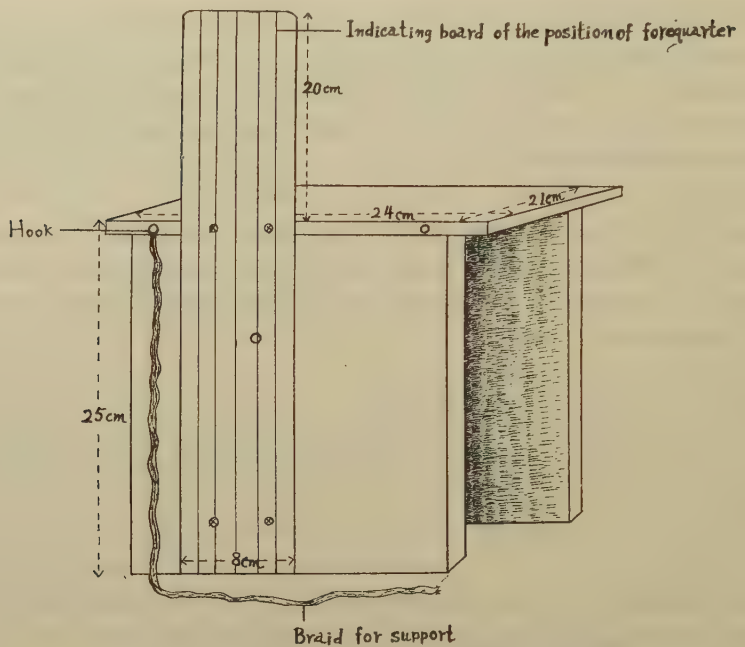
1. Application of Massage Method

The first method applied to the cock by BURROWS and QUINN (1935) was to massage the keel and the soft part of the abdomen above gizzard and below the pelvic bones of the bird which was held by an assistant standing opposite with its head downward. In applying similar massage method to the drake, ŌNISHI *et al.* (1950, 1955) kept the bird in sitting position on the ground by holding it firmly by the roots of the both wings with the left hand. With the right hand an irritable massage was repeatedly exerted upon the both thighs of the drake with the thumb on one side and the fore- and middle-fingers on the other.

On re-examining ŌNISHI's method, it was found that for drakes also the new method of BURROWS and QUINN (1937) using a bird holder was more convenient and successful. For this purpose WATANABE and SUGIMORI (1957) have newly developed the application of a drake holder. This holder consists of a small wooden table with a hight of 25 cm., a width of 21 cm. and a length of 24 cm. and a side-plate of 45 cm. in height and 8 cm. in width perpendicularly attached to it (Text-fig. 1). At manipulation the table is hung either on a wall or on a post. A drake is fixed on the table with its both wings and legs bound with a braid, the left side of the forequarter of the bird being situated along the side plate (Pl. II, Fig. 8, 9, 10). Thus, the position of the penis for operation can be ensured to come outside of the hind margin of the table (Pl. I, Fig. 1 & 2).

After the skin around the vent has been cleanly wiped with the sterilized physiological saline solution, the drake is stimulated on its both thighs (ilio-femoral regions) by rapidly massaging with the operator's right hand. This massage upon both thighs, however, is not massage in a real sense but quickly repeated flippings with the thumb on one side and the fore- and middle-finger on the other in ventro-dorsal direction from the acetabulum.

The first response to this massage is a stiffness of the penis in its original position and this is followed by a spasm of the whole body of the drake, specially of his neck and tail. At this moment, the stiff penis is to be pressed down for its complete evagination with the operator's right hand, then the ejaculate is caught into the receptacle which is beforehand kept beneath the cloaca with the operator's left hand (Pl. I, Fig. 3 & 4). The semen receptacle here employed is a glass cup of 5 cm. in diameter and 3 cm. in depth, its bottom being elongated into a graduated conical




Text-fig. 1. Drake holder for collecting semen.

tube of 12 cm. long (about 20 ml. content) (Pl. III, Fig. 13). The receptacle has to be sterilized before use.

Although an ejaculatory response seems to occur prior to this milking action, an ejaculate can be collected without loss into the receptacle because of long spiral passage of the ejaculatory groove of the penis.

The ilio-femoral massage thus applied to the drake does not vary in principle from the new method of BURROWS and QUINN (1937) applied to the cock, but because of morphological difference in the copulatory organs of the two birds, semen of the drake thus collected is less contaminated than that of the cock.

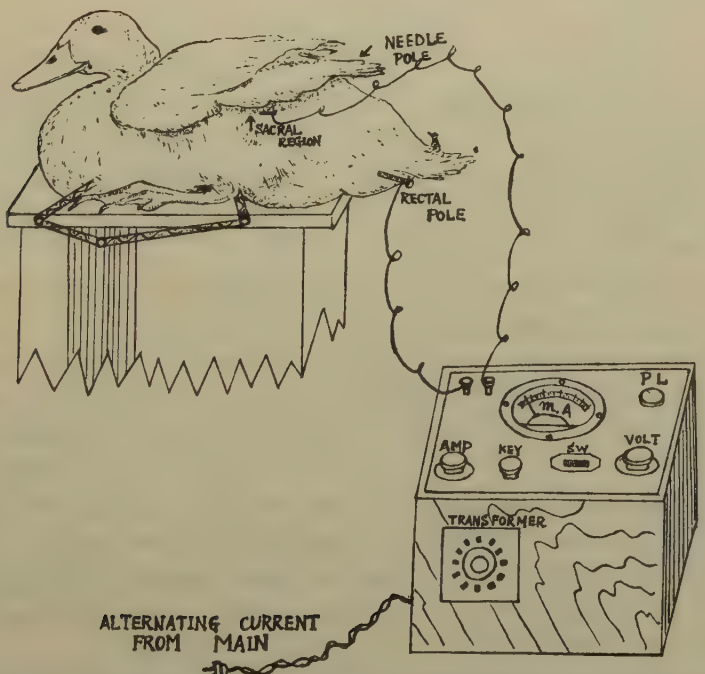
The time required for inducing an ejaculation by the massage is usually 30 seconds in the drakes well trained but cases were not seldom where an ejaculation occurred within 5–10 seconds. It is interesting to note that some trained drakes acquired a habit of erecting the penis or even ejaculating the semen before massage due to the conditioned reflex. Training of drakes, however, is fairly elaborate work as it takes usually a period of from 10 to 15 days until the bird shows an ejaculatory response to the massage. On the other hand, the newly trained drake requires much longer time, from 8 to 15 minutes, though this can be gradually shortened. As regards the individual difference in the ejaculatory response, of 47 drakes tested about 10 reacted to the massage without training, 24 needed training more or less and the remaining 13 did not react upon massage at all and the training had to be given up because of skin injuries effected by this repeated massage.

Semen always has to be collected early in the morning before supplying forages: 6 to 7 o'clock in summer and 7 to 8 o'clock in winter in order to avoid spoil-

ing of the semen with excrements. Further, just prior to the operation, the cloaca is pressed so that feces accumulated at the end of the rectum is expelled. Notwithstanding these precautions, it happened often that the semen thus procured had to be discarded because of soiling. It is also to be noticed that the semen volume here presented is not a volume in "physiological" sense, as it is hardly possible to measure the volume accurately because of its extremely small quantities.

2. Application of Electrical Stimulation

In the experiments of using the electrical method on drakes, an electro-ejaculator was employed. The apparatus was properly developed by SHIBATA *et al.* (1938) for applying to the rabbit, later commercially constructed by the FHK Co. Ltd., Tokyo, for applying a 60 cycles alternating current to the goat and sheep. The apparatus consists of a transformer, a voltmeter of 0–30 volts and a milliampmeter of 0–100 milliamps and a switch of push button (Pl. II, Fig. 5). In operation, an electric pole, a sharp needle which is led from the apparatus, was stuck into the hypodermis of the ilial region; and the opposite pole, a blunt rod, was inserted about 4 cm. deep into the rectum through the cloaca in the same manner as GUNN (1936) originally devised for the ram (Pl. II, Fig. 6; Text-fig. 2). At first the alternating current of 30 volts and 60–80 milliamps was turned on for 3 seconds and repeated by using a push button from three to five times with an interval of 5 seconds. Since the experiments with Muscovy drakes, as it will be later described, showed the cur-




Text-fig. 2. Diagram illustrating the method of electrical stimulation for collecting the semen of drake.

rent of 20 volts and 40–50 milliamps to be strong enough for inducing ejaculatory response, the same current was also applied subsequently to the Common duck with the same success. The operation occurred in the following way: A drake was first fixed on the bird-holder in the same manner as described in the massage method, then the current was turned on. At every turning of the current the drake showed a momentary rigidity of its body (Pl. II, Fig. 7 & 8). Upon sufficient stimulation, the bird flapped reflexively its tail revealing emission of semen from the vas deferens presumably into the urodeum (Pl. II, Fig. 9 & 10). It is peculiar to the electrical stimulation that the penis, contrary to the massage method, always remained in its original position without evaginating, though it showed some stiffness. For receiving the semen into a receptacle, therefore, further manipulation or "milking" was necessary in order to expel the semen from the urodeum and ejaculatory groove. This was simply done by pressing with fingers the penis somewhat stiffened in its original position. The time required for milking to induce the semen dropping into a receptacle was usually one minute. From the standpoint of avoiding contamination, it was more advantageous to recover the semen from the penis in its invaginated state than in its evaginated state.

It should not be left here unnoted that there is no evidence of injuries on the drakes treated with electricity of such a weak current as used here: Neither loss of appetite, nor decline of live weight, nor paralysis of body parts were observed. Ten birds subjected to the regular electrical stimulation during the past two years are producing normal semen still now.

## SUMMARY

1. The value of practical use of both the massage and electrical stimulation in collecting the semen from Common ducks was comparatively studied.

2. By using a drake-holder, the massage applied upon both the thighs of a drake induces easily an ejaculatory response. It is required, however, that the drake should be trained beforehand for about 10–15 days.

3. The repetition of massage for a long period of time gives sometimes injuries to the skin of the thighs operated upon.

4. As the reaction is partly of psychological nature, the length of time which is required from manipulation to ejaculation is fairly manifold: 50 to 5 seconds according to the training received. There are, however, some birds that do not react at all.

5. Application of an alternating current with 20 volts and 40–50 milliamps is strong enough to induce an ejaculatory response within one minute.

6. In the electrical stimulation, reaction being completely of reflexive nature, the collection of semen can be done with regular success. No deleterious effects has been found in birds thus treated.

7. The advantages and disadvantages of both techniques of semen collection shall be later discussed in association with other factors concerned.

## IV. CHARACTERISTICS OF SEMEN OF COMMON DUCKS

The semen which was collected by both the massage and electrical method was examined every time just after collection with respect to volume, sperm concentration, deformity percentage, pH-value and motility of sperms. Determination of sperm concentration was performed with a Thoma-Zeiss haemocytometer as usual. For determining the pH-values of semen the brom-thymol-blue test paper was used. The term deformity used here means a chain of the secondary changes sperm cells: looping of tail, bending or breaking at the middle piece, isolation of the head, etc.

### 1. Characteristics of Semen Collected by Massage Method

Massage method was applied to two drakes in the same season: April 25 to July 22, 1957. Microscopical examinations of semen carried out just after collection revealed that in the majority of cases, about 80–90 percent of sperms, were most active producing strong currents in semen drops, if the semen was collected in a clean state.

The results obtained from 6 samples of semen are summarized in Table I, in comparison with those secured by ŌNISHI *et al.* (1955), who have determined the per-ejaculate volume on 150 samples, and the per-unit-volume sperm concentration on 199 samples.

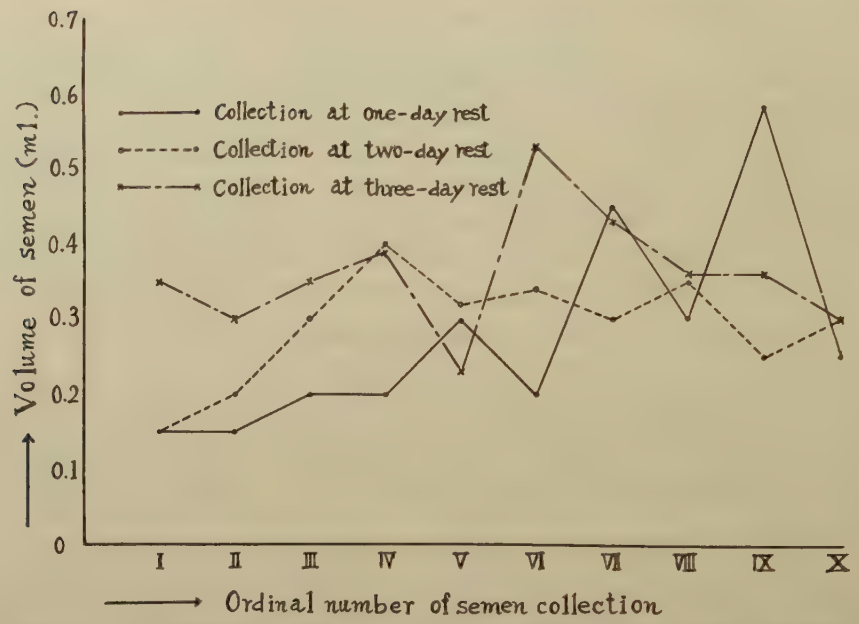
From the table it is seen that the semen collected by massage has an average volume of 0.233 ml. per ejaculate, an average number of 883,000 sperms per cubic millimeter, totalling 213.7 millions sperms per ejaculate. The average of pH-value of the semen is 6.73 and the average percentage of sperm deformity is 20.87. The first four figures of the average in the table show only slight differences from those determined by ŌNISHI *et al.* (1955), though the ranges are not always coincident.

In mammals, it has been known that semen volume and sperm concentration vary exceedingly at different intervals of collection, especially when this occurs repeatedly within a short time as in the twice-a-day collection. Since the duck receiving massage with such a frequency is very likely to sustain skin injuries, it is

Table I. Average volume, sperm concentration, pH-value and deformity percentage of the semen collected by massage per bird per collection.

| | WATANABE (1957 b) | | | ŌNISHI et al. (1955) | |
|---|---|---|---|---|---|
| | Mean | Range | Confidence limits(95%) | Mean | Range |
| Volume per ejaculate (ml.) | 0.233 | 0.05–0.45 | ±0.14 | 0.32 | 0.1–1.0 |
| Sperm concentration per cu. mm. (millions) | 0.883 | 0.340–1.360 | ±0.45 | 0.905* | 0.07–3.587* |
| Total number of sperms per ejaculate (millions) | 213.7 | 68.0–562.5 | ±2.09 | 263 | 1–1,550 |
| pH-value | 6.73 | 6.6–6.8 | ±0.10 | 7.31 | 6.6–7.8 |
| Sperm deformity (%) | 20.87 | 19.32–23.94 | ±1.66 | — | — |

* Converted from the number of sperms per ml. ejaculate.




Text-fig. 3. Relationship between the intervals of collection and the volume of semen.

desirable to determine the optimum interval of semen collections. In this connection, the figure cited from the previous paper (WATANABE & SUGIMORI, 1957a) may give some suggestions for it, if not conclusive. Each graph denotes an average of semen volume per bird per collection obtained from two drakes at a one-day rest, a two-day rest and a three-day rest (Text-fig. 3). In the trials where semen collections were made after a two-day or a three day-rest, the semen volume was greater than in the trial of a one-day rest, within a limited period of time, but when the collection occurred more continuously, the results showed a reverse tendency; it seemed to show that the bird can gradually adapt to massage and produce more semen after a one-day rest.

2. Characteristics of Semen Collected by Electrical Stimulation

The collection of semen by electrical stimulation was first performed with eight 2-year-old drakes during the season from April 25 to July 22, 1957, taking the above described two drakes as control to which massage was applied (WATANABE, 1957b). The results obtained are shown in Table II.

In comparing Tables II with Table I, most conspicuous fact is that both in semen volume and in sperm concentration the samples collected by electrical stimulation are always greater than those collected by massage. On account of technical difficulties of determining the exact volume below 1 ml., it is hardly possible to show any statistical significance for the semen volume, in consequence, also for the total number of sperm per ejaculate. Nevertheless, a significant difference in the sperm concentration per cubic millimeter is clearly found,

$$\text{revealing} \quad t_0 = 2.807 < t = 6.446$$

Table II. Characteristics of semen collected by electrical stimulation per bird per collection.

| | Mean | Range | Confidence limits (95%) |
|---|---|---|---|
| Volume per ejaculate (ml.) | 0. 326 | 0. 10–0. 75 | ±0. 08 |
| Sperm concentration per cu. mm. (millions) | 4. 624 | 2. 210–8. 660 | ±0. 89 |
| Total number of sperms per ejaculate (millions) | 1, 608. 2 | 442. 0–5, 196. 0 | ±6. 12 |
| pH-value | 6. 78 | 6. 4–7. 0 | ±0. 09 |
| Sperm deformity (%) | 20. 13 | 16. 06–23. 44 | ±0. 98 |

In fact, the semen obtained by electrical method appears unexceptionally thick and viscous in consistence with creamy color, whereas that obtained by massage is thin and less viscous and milky white in color.

In morphological character, the sperm cell of the duck seems apparently not to differ much from that of the fowl as shown in Text-fig. 4; the mean of the length of the heads and tails of 100 sperm cells measured were $15.02 \pm 0.469 \mu$ and $56.41 \pm 0.552 \mu$ respectively.

As regards the initial motility of sperms in the semen cellected by electrical stimulation, it may be said that the motility did not vary from the sample collected by massage.

Text-fig. 4. Sperm cells of Common duck.
Phase-contrast microscopy.
Magnification about 850.

Further experiments in semen collection by electrical stimulation were carried out on four 2-year-old drakes at every seven days for the period from April 1957 to March 1958 with the aim of its application to routine artificial insemination practice on one hand and, on the other hand, of examining the seasonal variation of semen characteristics.

The quantitative characters of semen thus obtained are shown in Table III in

Table III. Seasonal variation of characteristics of semen obtained by electric stimulation from Common duck (during the period from April 4, 1957 to March 27, 1958). Each number is an average of 4 ducks except those which are marked: One asterisk shows the case where only one drake responded to the stimulation; two, where two responded, and three, where three responded.

| Week No. | | Volume per ejaculate (ml.) | Sperm concentration per cu. mm. | Total number of sperms per ejaculate | Monthly average |
|---|---|---|---|---|---|
| | 1. | 0. 10 | 2, 640, 000 | 264, 000, 000 | |
| Jan. | 2. | 0. 10 | 3, 210, 000 | 321, 000, 000 | 246, 200, 000 |
| | 3. | 0. 10 | 1, 240, 000 | 124, 000, 000 | |
| | 4. | 0. 10 | 2, 760, 000 | 276, 000, 000 | |
| | 1. | 0. 20 | 4, 080, 000 | 816, 000, 000 | |
| Feb. | 2. | 0. 20 | 3, 120, 000 | 624, 000, 000 | 641, 500, 000 |
| | 3. | 0. 20 | 2, 490, 000 | 498, 000, 000 | |
| | 4. | 0. 20 | 3, 140, 000 | 628, 000, 000 | |
| | 1. | 0. 20 | 1, 348, 000 | 269, 600, 000 | |
| Mar. | 2. | 0. 30 | 8, 362, 000 | 2, 508, 600, 000 | 1, 607, 550, 000 |
| | 3. | 0. 20 | 16, 710, 000 | 3, 342, 000, 000 | |
| | 4. | 0. 20 | 1, 550, 000 | 310, 000, 000 | |
| | 1. | 0. 60 | 6, 460, 000 | 3, 876, 000, 000 | |
| Apr. | 2. | 0. 40 | 7, 900, 000 | 3, 160, 000, 000 | 3, 079, 000, 000 |
| | 3. | 0. 40 | 4, 620, 000 | 1, 848, 000, 000 | |
| | 4. | 0. 60 | 5, 720, 000 | 3, 432, 000, 000 | |
| | 1. | 0. 40 | 14, 100, 000 | 5, 640, 000, 000 | |
| May | 2. | 0. 30 | 6, 740, 000 | 2, 022, 000, 000 | 3, 570, 500, 000 |
| | 3. | 0. 50 | 7, 960, 000 | 3, 980, 000, 000 | |
| | 4. | 0. 40 | 6, 600, 000 | 2, 640, 000, 000 | |
| | 1. | 0. 20 | 2, 520, 000 | 504, 000, 000 | |
| Jun. | 2. | 0. 30 | 5, 140, 000 | 1, 542, 000, 000 | 1, 731, 250, 000 |
| | 3. | 0. 50 | 4, 270, 000 | 2, 135, 000, 000 | |
| | 4. | 0. 80 | 3, 430, 000 | 2, 744, 000, 000 | |
| | 1. | 0. 40 | 5, 580, 000 | 2, 232, 000, 000 | |
| Jul. | 2. | 0. 20 | 4, 850, 000 | 970, 000, 000 | 1, 126, 750, 000 |
| | 3. | 0. 10 | 7, 560, 000 | 756, 000, 000 | |
| | 4. *** | 0. 10 | 5, 490, 000 | 549, 000, 000 | |
| | 1. * | 0. 20 | 2, 620, 000 | 524, 000, 000 | |
| Aug. | 2. * | 0. 10 | 3, 390, 000 | 339, 000, 000 | 431, 500, 000 |
| | 3. | 0 | 0 | 0 | |
| | 4. | 0 | 0 | 0 | |
| | 1. ** | 0. 10 | 1, 240, 000 | 124, 000, 000 | |
| Sep. | 2. ** | 0. 10 | 2, 577, 000 | 250, 700, 000 | 159, 400, 000 |
| | 3. *** | 0. 10 | 1, 310, 000 | 131, 000, 000 | |
| | 4. | 0. 10 | 1, 250, 000 | 125, 000, 000 | |
| | 1. | 0. 10 | 2, 810, 000 | 281, 000, 000 | |
| Oct. | 2. | 0. 10 | 1, 570, 000 | 157, 000, 000 | 319, 750, 000 |
| | 3. | 0. 20 | 2, 890, 000 | 578, 000, 000 | |
| | 4. | 0. 10 | 2, 630, 000 | 263, 000, 000 | |
| | 1. | 0. 20 | 2, 800, 000 | 560, 000, 000 | |
| Nov. | 2. | 0. 20 | 3, 180, 000 | 636, 000, 000 | 524, 000, 000 |
| | 3. | 0. 10 | 3, 380, 000 | 338, 000, 000 | |
| | 4. | 0. 20 | 2, 810, 000 | 562, 000, 000 | |
| | 1. | 0. 10 | 3, 080, 000 | 308, 000, 000 | |
| Dec. | 2. | 0. 10 | 2, 840, 000 | 284, 000, 000 | 330, 750, 000 |
| | 3. | 0. 10 | 4, 170, 000 | 417, 000, 000 | |
| | 4. | 0. 10 | 3, 410, 000 | 314, 000, 000 | |

average values per bird per collection.

The table shows that the total number of sperms per ejaculate is at its highest during the period from the middle of March to the beginning of July, rising to a peak in May. A very conspicuous fact is that none of the drakes responded to the electrical stimulation in the last half of August although they were under the normal raising condition. Occasionally no response was also observed in another drakes over a period from the fourth week of July to the third week of September. Hence, this period appears to be the worst breeding season for the Common duck.

## SUMMARY

1. The results of examining 6 samples from 2 drakes showed that the average volume of semen collected by massage was 0.233 ml. per ejaculate, containing 883,000 sperms per cubic millimeter, thus totalling 213.7 millions sperms per ejaculate (Table I).

2. The average of 19 semen samples electrically collected from 8 drakes revealed 0.326 ml. in volume per ejaculate and 4,624,000 sperms per cubic millimeter, totalling 1,608.2 millions sperms per ejaculate (Table II).

3. Hence, the semen electrically collected is larger in its volume and about 5 times higher in sperm concentration than the semen collected by massage.

4. As a consequence, the total per ejaculate number of sperms is about 8 times bigger in the electro-ejaculated semen than in the semen procured by massage.

5. There were, however, no substantial difference in pH-value and deformity percentage of sperms between the semen collected by both the methods when examined immediately after collection (Table I and II).

6. Aside from the methods of collecting semen, a close relationship between the volume and frequency of collection of semen was found. A greater volume of semen was usually obtained at an interval of 2- or 3-day rest than at an interval of 1-day rest within a limited period of time. But when the collection occurred continuously for a longer period of time, the results appeared reversely.

7. A remarkable seasonal variation in sperm production was observed in a full-year record, showing a remnant of breeding season in wild life (Table III). A high testicular activity lasted from the middle of March to the beginning of July, rising to a peak in May. It declined, however, from the end of July to the third week of September, dropping off completely in the last half of August: the worst breeding season for the Common duck.

## V. TECHNIQUES OF SEMEN INTRODUCTION

Throughout present experiments, the insemination of ducks was carried out generally between 9 and 10 o'clock a. m. and at 11 o'clock a. m. at the latest. This relates, on one hand, to the time of semen collection before foraging the drakes, and on the other hand, to avoiding the new egg appearing in the uterus.

Introduction of semen into the oviduct of ducks was comparably tested by the

two methods: firstly by paipating the orifice and secondly by employing a speculum.

1. Techniques of Semen Introduction by Palpation

a. Experiments with semen collected by massage

Semen introduction by palpating method was applied to fifteen ducks in full lay which had been isolated from drakes for one month. Semen for the experiments was collected simultaneously from three drakes by massage. Every three samples thus collected were mixed immediately and each of the composite semen was diluted from 5 to 20 times according to the lot of experiments. To each duck, 0.3 ml. of the diluted composite semen was introduced into the uterus with the following procedure: After evacuating the bowel, a duck is held by an assistant sitting on a stool, the body of the bird being firmly held between both thighs of the assistant in order that the abdomen of the bird may be directed outwards and its vent upwards. An operator, after cleaning the vent with absorbent cotton, inserts his left index finger into it to search the vaginal orifice which can be palpated slightly shifting to the left at the upper part of the cloaca. As above mentioned, in the duck, the anterior part of the vagina leads to the uterus with a bend which can be felt by a weak pressure on the finger. The finger should be carefully inserted along its inner wall until it reaches the uterus.

Leaving the left finger at this position, a fine rubber tube with the length of about 10 cm. is introduced along it into the uterus by right hand. When the tip of the rubber tube reaches the uterus, the left finger is withdrawn and a common 1 ml. tuberculin-type syringe containing the semen is attached to the free end of the rubber tube and the content is slowly introduced. The rubber tube for the insemination of the rabbit used by YAMANE (1925) was found to be most satisfactory in this case, too.

The first experiment was performed on September 2, 1953 and the second on September 29, 1953. In both experiments the same birds were used, the insemination being done once per bird. The fertility of the semen thus introduced, was tested with eggs laid after the insemination, which were put into an incubator at 8.30 p. m. every evening and candled first on the 5th day of incubation. For the sake of confirmation, incubation was continued further for a week after the first candling. Tables IV and V show the laying records of the ducks in the two series of experiments, introducing the semen by palpation, and both these tables are combined into the Table VI by expressing in figures.

At a glance of these three tables it will be noticed that all the eggs produced on the first day following insemination are infertile and the first fertile eggs appear at the earliest on the second day after insemination.

In the duck, the time of onset of fertilization is not exactly known but the above data in association with the facts that the inseminations in the present study were always performed between 9 and 10 o'clock, and the eggs laid were collected between 6 and 7 o'clock every morning, it may be inferred that the eggs laid on the first day following insemination have no concern with the sperms introduced, but

Table IV. Laying record of 15 ducks inseminated with semen collected by massage and introduced by palpation (First experiment: September 2 to 16, 1953).

| No. day following insemination | No. duck inseminated | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 2 | − | − | − | + | + | + | − | + | + | + | 0 | − | + | + | + |
| 3 | − | − | − | + | + | + | − | + | + | + | + | − | + | + | + |
| 4 | − | − | − | + | + | + | − | + | + | + | − | − | + | + | + |
| 5 | − | − | − | + | + | + | − | + | + | + | + | − | + | + | + |
| 6 | − | − | − | + | + | − | − | + | − | + | + | − | + | 0 | + |
| 7 | − | − | − | + | + | − | − | + | − | − | − | − | + | + | + |
| 8 | − | − | − | 0 | + | − | − | − | − | − | + | − | 0 | − | + |
| 9 | − | − | 0 | 0 | + | − | − | + | − | + | − | − | − | − | − |
| 10 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 11 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 12 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 13 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 14 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |

Remarks: +=Fertile; −=Infertile; 0=No egg laid.

Table V. Laying record of the same 15 ducks as in Table IV, inseminated with semen collected by massage and introduced by palpation (Second experiment: September 29 to October 13, 1953).

| No. day following insemination | No. duck inseminated | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1 | 0 | − | 0 | − | − | − | − | − | − | 0 | − | − | − | 0 | − |
| 2 | − | + | 0 | + | + | + | − | 0 | + | + | + | + | 0 | + | + |
| 3 | − | + | 0 | + | + | + | + | + | + | + | + | + | 0 | + | + |
| 4 | 0 | + | 0 | 0 | 0 | − | + | 0 | + | + | + | + | 0 | + | + |
| 5 | 0 | + | 0 | + | 0 | + | − | + | 0 | − | − | − | + | + | + |
| 6 | 0 | + | 0 | + | 0 | − | − | + | + | − | − | − | − | 0 | + |
| 7 | + | + | 0 | + | + | − | − | − | + | − | − | + | 0 | − | − |
| 8 | + | + | − | + | + | − | − | 0 | − | − | − | 0 | − | − | + |
| 9 | − | + | 0 | + | + | + | − | + | + | − | − | − | − | + | + |
| 10 | + | + | 0 | + | − | − | − | − | − | − | − | − | − | − | − |
| 11 | − | − | 0 | + | − | − | − | − | − | − | − | − | − | − | − |
| 12 | 0 | − | 0 | + | + | − | − | − | − | − | − | − | − | − | − |
| 13 | − | − | 0 | − | − | − | − | − | − | − | − | − | − | − | − |
| 14 | − | − | 0 | − | − | − | − | − | − | − | − | − | − | − | − |

Remarks: +=Fertile; −=Infertile; 0=No egg laid.

the fertilization should occur just on that day. It follows, therefore, that the eggs produced on the first day after insemination should not be considered in calculating the fertility rate. On the same account, the duration of the fertilizing capacity of

Table VI. Fertility test of semen collected by massage and injected to 15 ducks by palpation (Compiled from Table IV and V).

| No. day following insemination | Number of egg laid | | | Number of fertile eggs | | | *Daily fertility (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp. I | Exp. II | | Exp. I | Exp. II | | Exp. I | Exp. II | Average |
| 1 | 15 | 11 | | 0 | 0 | | 0 | 0 | 0 |
| 2 | 14 | 12 | | 9 | 10 | | 64.3 | 83.3 | 73.8 |
| 3 | 15 | 13 | | 10 | 12 | | 66.7 | 92.3 | 84.5 |
| 4 | 15 | 9 | | 9 | 8 | | 60.0 | 88.9 | 75.0 |
| 5 | 15 | 11 | | 10 | 7 | | 66.7 | 63.6 | 65.1 |
| 6 | 14 | 11 | | 7 | 5 | | 50.0 | 45.5 | 47.8 |
| 7 | 15 | 13 | | 6 | 6 | | 40.0 | 46.2 | 43.1 |
| 8 | 13 | 13 | | 3 | 5 | | 23.1 | 38.5 | 30.8 |
| 9 | 13 | 14 | | 3 | 8 | | 23.1 | 57.1 | 40.1 |
| 10 | 14 | 14 | | 0 | 3 | | 0 | 21.4 | 10.7 |
| 11 | 15 | 14 | | 0 | 1 | | 0 | 7.1 | 3.6 |
| 12 | 15 | 13 | | 0 | 2 | | 0 | 15.4 | 7.7 |
| 13 | 15 | 14 | | 0 | 0 | | 0 | 0 | 0 |
| 14 | 15 | 14 | | 0 | 0 | | 0 | 0 | 0 |
| | | 114 | 137 | | 57 | 67 | | | |

* In testing the fertilizing capacity of the semen of poultry, the fertility rate may be calculated from two aspects as reported in earlier papers (WATANABE and SUGIMORI, 1957 a; WATANABE, 1957 b): the rate of the number of birds inseminated and produced fertile eggs to the number of all birds inseminated, viz. absolute fertility, and the rate of the number of fertile eggs to the total number of eggs produced per bird per insemination, viz. relative fertility. Since it has been subsequently found that the use of the fertility rate per bird or per flock per day or per week or per some days is more rational for judging the fertility of semen samples inseminated, this term will be used hereafter.

sperms may be indicated by the day before the last fertile egg has been laid. Considering the approximate time of onset of fertilization as above mentioned, further analysis of the data presented in the Table VI reveals that the fertility rate of eggs laid during the period from the second day following insemination to the day producing the last fertile egg is 50.0 percent (57: 114) in the first experiment, and 48.9 percent (67: 137) in the second experiment; thus the results of both experiments was almost identical.

It is peculiar, however, to find the less fertilizing capacity of semen in the first experiment as compared to that in the second experiment, manifesting clearly in the daily fertility rates and the durations of retaining fertility potential. In this connection, it must be remembered that the first experiment was performed during the first half of September when the semen qualities were inferior as has been indicated in the foregoing chapter. On the contrary, the second experiment was conducted during the first half of October when the semen was already improve quantitatively as well as qualitatively.

b. Experiments with the semen collected by electrical stimulation

As it has been shown, the semen collected by electrical method is strikingly

thick compared with that collected by massage, and the introduction of the semen electrically obtained should be also tested. All the treatments of the semen did not differ from the preceding experiment, except that it was diluted only 10 times in spite of its greater density.

Ten ducks were used for this experiment; the results obtained are shown in Table VII.

Table VII. Fertility test with semen electrically collected and introduced to 10 ducks by palpation (May 19 to June 3, 1957).

| No. day following insemination | Number of eggs laid | Number of fertile eggs | Daily fertility (%) |
|---|---|---|---|
| 1 | 8 | 0 | 0 |
| 2 | 8 | 4 | 50.0 |
| 3 | 8 | 5 | 62.5 |
| 4 | 7 | 2 | 28.6 |
| 5 | 7 | 3 | 42.9 |
| 6 | 6 | 2 | 33.3 |
| 7 | 4 | 1 | 25.0 |
| 8 | 5 | 2 | 40.0 |
| 9 | 1 | 0 | 0 |
| 10 | 3 | 0 | 0 |
| 11 | 2 | 0 | 0 |
| Total number of eggs from 2 to 8 day after insemination | 45 | 19 | 7-day fertility (%) 42.2 (19:45) |

The first fertile eggs appeared on the second day after insemination and the daily maximum fertility rate was gained on the third day as shown in the foregoing experiment.

It is worthy of note that the electro-ejaculated semen exhibited a lower fertility than that collected by massage. This, however, is not likely to be attributed to the introducing technique, but rather to the characteristics of the semen collected by electrical stimulation. The matter will be discussed below.

2. Techniques of Semen Introduction by Employing a Speculum

In the artificial insemination of mammals, a vaginal speculum is of common use, but no attempt has been made of its use for birds; this may be due to the smallness and peculiar form of the cloaca. In the present experiments, however, it was shown that the speculum is easily applicable to the duck and very useful. A metallic speculum was devised, approximating the anatomical structure of copulatory organs of the duck. The part to be inserted of the speculum is 7 cm. in length, 1.3 cm. in depth and 1.5 cm. in width and its handle bends at 145°, and measures 14 cm. in direct length; the bend of the handle firmly lets the speculum fix on the operator's index finger (Pl. III, Fig. 11 & 12). For semen introduction, in this case,

a glass pipette of 10 cm. in length and 0.3 ml. content which was connected to a rubber bulb at its hind end was used. The bird was held in a similar manner by an assistant and cleaned at its vent as described before.

On inserting the speculum through the vent, the firmly closed anal opening can be found at the center of anterior wall of the cloaca. Slightly shifting from the anal opening to the upper left lies the vaginal orifice loosely closing. Further manipulation with the speculum easily enables the insertion of it about 7 cm. deep through the vaginal orifice. Hereupon, the insemination pipette sterilized beforehand is introduced along the speculum until its tip reaches the fore-end of the speculum, and the content is injected by pressing the rubber bulb attached (Pl. III, Fig. 13–16).

The whole procedure from insertion of the speculum to the semen introduction was only one minute's work; insemination could be performed quickly without worry of bringing injury or infection to the female birds.

A further fertility test was made as before in a flock of another 10 ducks from February 4 to 21, 1957. The result shown in Table VIII indicates slightly higher fertility rates than those found in the case of semen introduction by palpation method (Table VII & VIII). Hence, there is no reason to believe that at semen introduction the palpation method is more preferable to the use of speculum.

Table VIII. Fertility test with semen electrically collected and introduced by applying speculum in a flock of 10 ducks (February 4 to 21, 1957).

| No. day following insemination | Number of eggs laid | Number of fertile eggs | Daily fertility (%) |
|---|---|---|---|
| 1 | 9 | 0 | 0 |
| 2 | 10 | 8 | 80.0 |
| 3 | 10 | 5 | 50.0 |
| 4 | 9 | 5 | 55.6 |
| 5 | 9 | 2 | 22.2 |
| 6 | 9 | 1 | 11.1 |
| 7 | 10 | 1 | 10.0 |
| 8 | 9 | 0 | 0 |
| 9 | 9 | 0 | 0 |
| 10 | 9 | 0 | 0 |
| Total number of eggs laid from 2 to 8 day after insemination | 57 | 22 | 6-day fertility (%) 38.6 (22:57) |

## SUMMARY

1. In all cases of the present experiments, semen introduction was carried out between 9 to 10, rarely 11 o'clock a. m., considering the time of semen collection as well as the time of forming the egg-shell within the oviduct.

2. For introducing semen to the oviduct the two techniques, viz., palpation

method and method employing a speculum, were compared, both the semen collected electrically and by massage being used.

3. Semen diluted with the physiological saline was introduced into the uterus by palpating the vaginal orifice with the index finger on one hand, and by inserting a speculum through the vaginal orifice, on the other.

4. The palpation method with semen obtained by massage (Exp. a), brought a result that the maximum fertility was obtained on the third day after insemination, showing the fertility of 66.7 and 92.3 percent with the average of 84.5 percent. The maximum period during which the sperm maintained its fertility was 11 days.

5. By the palpation method with the semen obtained by electrical stimulation, the daily fertility rate dropped to 62.5 percent at maximum and the duration of maintaining fertility was shortened to 7 days (Exp. b). This is, however, unlikely to be attributed to the introducing technique itself as discussed below.

6. The use of a speculum specially devised for the purpose of semen introduction was found to be decidedly advantageous. It could be performed quickly without giving any damage to ducks.

7. The fact that the fertility rates of semen electrically obtained for both experiments by palpation method and employing a speculum was lower than that of semen secured by massage, seem to indicate the proper characteristics of the semen collected by electrical stimulation. The matter is considered below.

## VI. EFFECTS OF DILUTORS AND STORAGE ON FERTILITY OF DUCK SEMEN

### 1. Effects of Dilution Rates

In the fowl, the relationship between the dilution rate and the fertility of semen have been reported by a number of workers but there is very little information available on the duck.

In the present experiments, the semen collected by massage from 4 drakes was added with 0.85 percent physiological saline solution, each sample being subjected to the dilution rates of 1:0, 1:5, 1:10 and 1:20. Each semen thus diluted was fractionally inseminated to five ducks, with a dose of 0.3 ml. per bird. All eggs laid after the insemination were incubated for fertility test. Table IX indicates clearly the beneficial effect of dilution but within a limited extent.

While the undiluted semen showed 100 percent fertility until the third day after the insemination, the semen diluted with a rate of 5, 10 and 20 times resulted 100 percent fertility until the seventh, third and fourth day, respectively, and most satisfactory fertility level was maintained in the 5-time dilution. As these resultant fertility rates have been obtained by a single insemination per bird, it should be inferred that the most satisfactory fertility level will be continuously retained, if the insemination is periodically performed with a cycle of every five days by using a fresh semen.

Table IX. Relationships between the dilution rate and the fertility of semen obtained by massage and diluted with physiological saline solution (September 2 to October 13, 1953). Sperm suspension of each rate was alloted to five ducks in a volume of 0.3 ml. per bird.

| No. day following insemination | Number of eggs laid | | | | Number of fertile eggs | | | | Daily fertility (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dilution rate | | | | Dilution rate | | | | Dilution rate | | | |
| | 0 | 5 | 10 | 20 | 0 | 5 | 10 | 20 | 0 | 5 | 10 | 20 |
| 1 | 5 | 3 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 100 | 75 | 75 | 100 |
| 3 | 4 | 4 | 5 | 4 | 4 | 3 | 5 | 4 | 100 | 75 | 100 | 100 |
| 4 | 5 | 1 | 4 | 3 | 3 | 1 | 3 | 3 | 60 | 100 | 75 | 100 |
| 5 | 5 | 2 | 4 | 5 | 4 | 2 | 2 | 3 | 80 | 100 | 50 | 60 |
| 6 | 5 | 2 | 4 | 4 | 4 | 2 | 2 | 1 | 80 | 100 | 50 | 25 |
| 7 | 5 | 4 | 5 | 4 | 3 | 4 | 1 | 1 | 60 | 100 | 20 | 25 |
| 8 | 4 | 5 | 4 | 4 | 3 | 4 | 0 | 1 | 75 | 80 | 0 | 25 |
| 9 | 5 | 4 | 5 | 5 | 2 | 3 | 3 | 2 | 40 | 75 | 60 | 40 |
| 10 | 4 | 4 | 5 | 5 | 1 | 3 | 0 | 0 | 25 | 75 | 0 | 0 |
| 11 | 3 | 4 | 5 | 5 | 1 | 1 | 0 | 0 | 33.3 | 25 | 0 | 0 |
| 12 | 4 | 3 | 5 | 5 | 0 | 2 | 0 | 0 | 0 | 66.7 | 0 | 0 |
| 13 | 5 | 4 | 5 | 5 | 1 | 0 | 0 | 0 | 20 | 0 | 0 | 0 |
| 14 | 4 | 4 | 4 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 4 | 4 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

2. Effects of Egg-yolk Citrate Buffers

In all the experiments above mentioned, 0.85 percent saline solution was used as dilutor of semen, because no adequate dilutor so far had been known for duck semen. Nevertheless, the physiological saline solution is not always suitable dilutor in keeping the fertility of sperm for a long period of time *in vitro*. In order to promote fertility of the sperms, regardless of immediate use after collection or long-term storage *in vitro*, the preparation of an expedient dilutor is indispensable.

In the present experiments three kinds of egg-yolk buffers were provisionally used:

1. Fowl egg-yolk citrate solution consisting of 3g $Na_3C_6H_5O_7 \cdot 2H_2O$ dissolved in 100 ml. distilled water and 100 g fresh fowl egg-yolk,
2. Duck egg-yolk citrate solution prepared in the same ratio as above, and
3. "Seminan", a commercial fowl egg-yolk citrate buffer devised by NISHIKAWA (1954) for diluting the bull semen.

Semen samples for the test were simultaneously collected by electrical method from three 8-month-old drakes and the three semen samples were mixed and put into 5 vials in an equal proportion; each content of the vials was diluted five times with the dilutors above mentioned. After dilution, all the samples were gradually cooled to 5°C within half an hour, then stored at this temperature in a refrigerator. Every 24 hours, sperm motility was examined under the microscope equipped with a stage thermostat. During the first 24 hours after collection each semen sample showed very active motility of sperm, regardless of dilution or non-dilution or kinds

of dilutors; the difference in the viability, expressed by the maximum duration of motility, occurred from the second day on. The results of the experiments with 8 lots are summarized in Table X.

It is seen that, compared with the undiluted semen, all dilutors here tested were more or less effective for the viability of sperm, but among them the fowl egg-yolk citrate was decidedly effective. It was noteworthy that the duck egg-yolk citrate buffer and "Seminan" were less effective than the simple saline medium.

Table X. Average viability of duck sperms in various dilutors.

| | Mean (hrs.) | Range (hrs.) | Confidence limits (95%) |
|---|---|---|---|
| Undiluted | 36 | 24—72 | ±12.63 |
| Physiological saline solution (0.85%) | 78 | 48—96 | ±20.72 |
| Fowl egg-yolk citrate | 126 | 48—192 | ±41.11 |
| Duck egg-yolk citrate | 63 | 24—96 | ±21.22 |
| "Seminan" | 60 | 24—96 | ±21.41 |

3. Storage and Fertility of Semen in Egg-Yolk Buffer

Based on the foregoing result that the fowl egg-yolk citrate solution was most effective for maintaining the motility of semen, further experiments on the insemination with the semen stored in this solution were made to a greater extent during the period from November 23, 1959 to March 4, 1960.

Semen was collected electrically; a composite sample from five 8-month-old drakes was diluted 5 times with the fowl egg-yolk citrate buffer, gradually cooled to 5°C within half an hour and stored in a refrigerator at this temperature as before described. Thirty ducks of 8-months of age were divided into 5 flocks, each consisting of 6 ducks (Flock I to V). Each buffered semen sample was injected simultaneously to each flock with a dose of 0.3 ml. per bird. Fertility test was made with the semen stored for 0, 24, 48, 72 and 96 hours but the results of the last two tests were quite negative, no fertile egg being produced; thence the results obtained in Flock IV and V were discarded here (Table XI).

In the Flock I where the buffered semen was injected immediately after collection, the most satisfactory fertility rate was gained until the 5th day following insemination and the maximum duration of fertility was 13 days. Considering that this was the result of a single insemination will be repeated every five days, a full fertility can be gained in practice. In the case of the Flocks II and III tested with semen stored for 24 and 48 hours respectively, the duration of fertility was remarkably short. These cases also show that, a repeated insemination with a cycle of every three days would result in 80 percent fertility or more. A longer storage than 48 hours has little likelihood of retaining fertility potential under the present condition, although about two-thirds of sperms in the microscopical field exhibited

Table XI. Fertility of the semen electrically collected and stored in a fowl egg-yolk citrate buffer at dilution rate of 1:5. Insemination took place once for each flock, consisting of 6 ducks.

| No. day following insemination | Flock I. 0-hour storage | | | Flock II. 24-hour storage | | | Flock III. 48-hour storage | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of eggs laid | Number of fertile eggs | Daily fertility (%) | Number of eggs laid | Number of fertile eggs | Daily fertility (%) | Number of eggs laid | Number of fertile eggs | Daily fertility (%) |
| 1 | 3 | 0 | 0 | 5 | 0 | 0 | 4 | 0 | 0 |
| 2 | 6 | 4 | 66.7 | 6 | 3 | 50.0 | 5 | 4 | 80.0 |
| 3 | 6 | 6 | 100.0 | 6 | 5 | 83.3 | 5 | 4 | 80.0 |
| 4 | 4 | 3 | 75.0 | 6 | 2 | 33.3 | 3 | 1 | 33.3 |
| 5 | 4 | 4 | 100.0 | 5 | 2 | 40.0 | 5 | 3 | 60.0 |
| 6 | 4 | 2 | 50.0 | 6 | 2 | 33.3 | 5 | 1 | 20.0 |
| 7 | 6 | 2 | 33.3 | 6 | 2 | 33.3 | 6 | 0 | 0 |
| 8 | 6 | 2 | 33.3 | 5 | 2 | 40.0 | 6 | 0 | 0 |
| 9 | 5 | 1 | 20.0 | 6 | 2 | 33.3 | 6 | 0 | 0 |
| 10 | 3 | 1 | 33.3 | 6 | 1 | 16.7 | 4 | 0 | 0 |
| 11 | 4 | 2 | 50.0 | 6 | 0 | 0 | 6 | 0 | 0 |
| 12 | 6 | 1 | 16.7 | 6 | 0 | 0 | 6 | 0 | 0 |
| 13 | 6 | 1 | 16.7 | 6 | 0 | 0 | 6 | 0 | 0 |
| 14 | 4 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |

very active motion at the time of insemination. The fertilizing capacity of the stored semen can be measured neither by motility nor viability as PARKER, MCKENZIE and KEMPSTER (1942) and WILCOX and SHAFFNER (1957) have already pointed out.

## SUMMARY

1. The beneficial effects of dilution were clearly shown. In the 5-time dilution of semen a full fertility appeared until the 7th day after insemination, whereas this lasted only for 3 days in the undiluted semen.

2. Further dilution than 5 times lowers the fertility rate; this may be due to the excessive reduction of sperm number.

3. Three kinds of egg-yolk citrate buffer were compared with the physiological saline in respect to the fertilizing capacity of stored semen.

4. In 5-time dilution the best result was obtained from the semen buffered with fowl egg-yolk citrate.

5. In this buffer the semen injected immediately after collection showed 100 percent daily fertility with a slight intermediate dropping from the third to fifth day following insemination. The fertility potential was maintained until the twelfth day after insemination (Table XI, Flock I).

6. The semen of 24-hour storage showed 83.3 percent fertility on the third day following insemination; the fertility potential was maintained until the ninth day (Table XI, Flock II).

7. The daily fertility of the semen stored for 48 hours was almost the same

as that of the semen stored for 24 hours. However, the duration of the fertility was quite different; it was maintained only for 5 days in the case of 48-hour storage (Table XI, Flock III).

8. Considering that only little is known about the successful storage of avian semen, the above findings about 24- and 48-hour storage times should be considered very promising for practical purpose.

9. The problem of maintaining a full fertility level will be easily solved by a periodical insemination with a cycle of five days in the case of immediate use of semen after collecting and with a cycle of three days in the case of 24- or 48-hour storage.

10. Further extension of the cycle of periodical insemination will be possible by developing a more suitable buffer.

## VII. APPLICATION OF ARTIFICIAL INSEMINATION FOR MULE-DUCK PRODUCTION

The mule-duck is a hybrid bird produced by mating of the Common duck with the Muscovy drake. In spite of its excellent meat qualities, its production is not a little impeded because of the low fertility of intergeneric hybrid eggs. According to a report of the Taichung Animal Industry Association, Taiwan (1943), of 4,952 hybrid eggs incubated only 2,094 eggs were fertile, showing a fertility of 42.3 percent. This partial infertility has been believed by some people of Taiwan to be caused by a difference in mating behavior of the parent species: the Common duck has a water-copulatory habit and the Muscovy, a land-copulatory habit.

On account of the importance of mule-duck raising from the practical point of view, the experiments of artificial insemination for mule-duck production were undertaken with the aim of promoting the fertility of intergeneric hybrid eggs.

The birds used for the present experiments consisted of an adult Colored Muscovy drake and five 2-year-old Common ducks. While this Muscovy drake was that raised in Okayama Prefecture, the Common ducks were of the same strain of Osaka breed as used in the foregoing experiments. Besides the said drake, 5 males and 3 females of Muscovy ducks, raised in the author's laboratory pens, were subjected to subsequent observations. They were secured by incubating either the eggs transported by air from Taipei, Taiwan, or the eggs produced in this laboratory. The initial eggs were kindly presented by Dr. E. RYU, Professor of The College of Agriculture, National Taiwan University, Taipei, Taiwan, to whom the auther wishes to express his heart-felt thanks on this occasion.[1)]

### 1. Copulatory Behavior of Muscovy Drake in Mating with Common Duck

The average live weight of the Common duck used in the present experiments was 2.3 kg in the male and 1.8 kg in the female. On the other hand, the Muscovy

1) Of 22 eggs shipped from Taiwan 5 samples were damaged by transportation. No data on the time of laying were accessible but 17 eggs incubated resulted 35.2 percent fertility (6: 17), 66.7 percent hatchability (4: 6) and 33.3 percent embryonic mortality (2: 6).

drake here used for mating showed the live weight of 4.2 kg which was much heavier than the average value of $3,752.50 \pm 146.86$ g given by SOKOLOVSKAJA (1935) (Pl. IV, Fig. 17 and 18).

The Muscovy drake exhibited much stronger sexual activity against the Common duck than the Common drake but a great difference both in body size and weight is likely to cause a copulatory hindrance. The Muscovy drake mounting on the back of the Common duck has to stabilize his body with great effort though the duck showed her receptivity. This would make the burden too heavy for the duck sitting on ground instead of floating on water. As a matter of fact, the Muscovy drake, upon ejaculating, falls down aside the duck without withdrawing the penis from the duck's body, until the latter escapes from the former, stands up and cleans her body with her bill. Unlike the Common duck, the Muscovy drake standing up later runs to a basin to clean his body. In all cases, so far no copulation was observed ending without ejaculatory response.

2. Collection of Semen from Muscovy Drake

Unlikely the Common drake, the Muscovy drake hardly shows an ejaculatory response to massage. This is partly due to the lack of docility of the Muscovy drake and also to too much breadth between both the thighs of this bird to apply the massage. Therefore, in the present experiment, the electrical method had to be employed for the collection of semen.

The same procedures as those described for the Common drake were taken except that a weaker current was applied: 20 volts and 40–50 milliamps in place of 30 volts and 60–80 milliamps. In this case also, the electrical stimulation could be applied without any deleterious effects to the bird: the drake has been used for the semen collection for these three years and still it is being used.

Semen was regularly collected once a week from December 5, 1957 to November 30, 1958 in order to find out if there is any seasonal variation as well as to use for occasional insemination.

3. Characteristics of Muscovy Semen

Every ejaculate was examined immediately after collecting in respect to its characteristics. Generally speaking, the semen in spite of electrical collection, was very thin and appeared milky white in color, similarly to the semen of the Common duck collected by massage. At examination just after collection, about 85 percent of sperms always exhibited very active motility.

Table XII gives a record of the characteristics of Muscovy semen examined throughout the year.

A certain seasonal variation of the semen characteristics of the Common duck has been shown above (Table III). In the case of the Muscovy also, the similar variation was found. The total number of sperm per ejaculate is greatest in June, and smallest in November and December, although the curve, if drawn, will never be smooth. In comparing the Table XII with the Table III obtained from the Common duck, the most conspicuous fact is that, of the Muscovy semen, the sperm

Table XII. Seasonal variation of characteristics of Muscovy semen obtained electrically (from December 5, 1957 to November 30, 1958).

| Week No. | | Volume per ejaculate (ml.) | Sperm cocentration per cu. mm. | Total number of sperms per ejaculate | Monthly average |
|---|---|---|---|---|---|
| Jan. | 1. | 0.18 | 599,000 | 107,820,000 | 77,368,000 |
| | 2. | 0.35 | 305,000 | 106,750,000 | |
| | 3. | 0.32 | 220,000 | 70,400,000 | |
| | 4. | 0.35 | 70,000 | 24,500,000 | |
| Feb. | 1. | 0.35 | 430,000 | 150,500,000 | 99,625,000 |
| | 2. | 0.50 | 190,000 | 95,000,000 | |
| | 3. | 0.20 | 90,000 | 18,000,000 | |
| | 4. | 0.30 | 450,000 | 135,000,000 | |
| Mar. | 1. | 0.50 | 240,000 | 120,000,000 | 100,780,000 |
| | 2. | 0.30 | 300,000 | 90,000,000 | |
| | 3. | 0.20 | 380,000 | 76,000,000 | |
| | 4. | 0.32 | 366,000 | 117,120,000 | |
| Apr. | 1. | 0.35 | 32,000 | 11,200,000 | 13,950,000 |
| | 2. | 0.30 | 22,000 | 6,600,000 | |
| | 3. | 0.40 | 45,000 | 18,000,000 | |
| | 4. | 0.40 | 50,000 | 20,000,000 | |
| May | 1. | 0,20 | 61,000 | 12,200,000 | 51,850,000 |
| | 2. | 0.60 | 114,000 | 68,400,000 | |
| | 3. | 0.50 | 64,000 | 32,000,000 | |
| | 4. | 0.60 | 158,000 | 94,800,000 | |
| Jun. | 1. | 0.60 | 330,000 | 198,000,000 | 154,300,000 |
| | 2. | 0.70 | 298,000 | 208,600,000 | |
| | 3. | 0.30 | 134,000 | 40,200,000 | |
| | 4. | 0.60 | 284,000 | 170,400,000 | |
| Jul. | 1. | 0.20 | 208,000 | 41,600,000 | 40,800,000 |
| | 2. | 0.40 | 96,000 | 38,400,000 | |
| | 3. | 0.50 | 80,000 | 40,000,000 | |
| | 4. | 0.40 | 108,000 | 43,200,000 | |
| Aug. | 1. | 0.30 | 236,000 | 70,800,000 | 81,550,000 |
| | 2. | 0.50 | 294,000 | 147,000,000 | |
| | 3. | 0.30 | 142,000 | 42,000,000 | |
| | 4. | 0.40 | 166,000 | 66,400,000 | |
| Sep. | 1. | 0.40 | 158,000 | 63,200,000 | 90,300,000 |
| | 2. | 0.50 | 102,000 | 51,000,000 | |
| | 3. | 0.50 | 218,000 | 109,000,000 | |
| | 4. | 0.30 | 460,000 | 138,000,000 | |
| Oct. | 1. | 0.30 | 80,000 | 24,000,000 | 13,750,000 |
| | 2. | 0.20 | 4,000 | 800,000 | |
| | 3. | 0.30 | 96,000 | 28,800,000 | |
| | 4. | 0.10 | 14,000 | 1,400,000 | |
| Nov. | 1. | 0.10 | 20,000 | 2,000,000 | 1,025,000 |
| | 2. | 0.30 | 3,000 | 900,000 | |
| | 3. | 0.10 | 10,000 | 1,000,000 | |
| | 4. | 0.20 | 1,000 | 200,000 | |
| Dec. | 1. | 0.20 | 35,000 | 7,000,000 | 8,075,000 |
| | 2. | 0.10 | 83,000 | 8,300,000 | |
| | 3. | 0.05 | 128,000 | 6,400,000 | |
| | 4. | 0.05 | 212,000 | 10,600,000 | |

number both per cubic millimeter and per ejaculate are strikingly smaller than those of the Common duck, although the semen volume in the former is apparently larger than in the latter; in fact, Muscovy semen shows macroscopically much thinner consistency. In maximum sperm concentration, the Muscovy semen is about 28 times smaller than that of the Common duck (Jan. 1st wk. in Table XII and March 3rd wk. in Table III), and the Muscovy is about 23 times inferior to the Common duck in its sperm productivity at their respective peaks on monthly average (June-average in Table XII and May-average in Table III).

4. Insemination of Common Ducks with Muscovy Semen

Inseminating experiments with the aim of hybridizing Common ducks with a Muscovy drake were conducted on September 23, 1958, though it was an unfavorable breeding season for ducks as seen above in the sperm productivity. The results obtained from the inseminations in a worse breeding season, however, may give expectations of gaining better fertility rates, if these would be done in a better breeding season.

An ejaculate recovered from a Muscovy drake was alloted for insemination to five Common ducks which were beforehand proved to be good layers and strictly isolated from drakes. The semen secured was immediately diluted by 10 times with 0.85 percent physiological saline solution and each 0.3 ml. of this mixture was simultaneously injected into the five ducks. Since the total volume of the ejaculate was 0.5 ml. containing 102,000 sperms per cubic millimeter, the total number of sperms each duck received was 3,060,000, each sample containing 10,200 sperms per cubic millimeter. At the microscopical examination of semen immediately after collection, about 85 percent of sperms exhibited very active motility. The introduction of the semen was made by employing a speculum.

All the eggs laid during two weeks following the insemination were tested by putting into an incubator at 10.00 a. m. every day and the eggs verified to be fertile by candlings were further incubated to hatching.

The total number of hybrid eggs produced by the five ducks during a week following the insemination were 28 consisting of 20 infertile and 8 fertile eggs. Since the onset of fertilization takes place on the second day following insemination, 3 eggs laid on the first day following the insemination should be eliminated from accounting the fertility of introduced semen. If so be, the 6-day fertility rate at a single insemination for this flock will be 32.0 percent (8:25). Table XIII gives the results of insemination with the heterologous semen accompanying the fertility percentage per bird per day.

The Muscovy semen released in the oviduct of the Common duck kept fertility for 6 days at most, which did not differ from the insemination with the homologous semen (cf. Table VIII). However, as far as the daily fertility rate was concerned, it appeared that the semen of these two duck species were not always identical in physiological efficiency. On the second day following the insemination when the maximum fertility rate was expected, the heteroinsemination showed 75 percent fertility whereas it was 80 percent in the homoinsemination. On the fourth day it

Table XIII. Results of simultaneous inseminations of 5 Common ducks with fractions of a Muscovy ejaculate electrically obtained. The semen was diluted 10 times with physiological saline solution (September 23, 1958).

| No. day following insemination | No. ducks inseminated | | | | | Number of eggs laid | Number of fertile eggs | Daily fertility (%) |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | |
| 1 | 0 | – | 0 | – | – | 3 | 0 | 0 |
| 2 | 0 | + | + | – | + | 4 | 3 | 75.0 |
| 3 | + | – | + | – | 0 | 4 | 2 | 50.0 |
| 4 | + | – | + | 0 | – | 4 | 2 | 50.0 |
| 5 | – | – | – | – | – | 5 | 0 | 0 |
| 6 | – | 0 | 0 | – | – | 3 | 0 | 0 |
| 7 | – | – | + | – | – | 5 | 1 | 20.0 |
| 8 | 0 | 0 | 0 | – | 0 | 1 | 0 | 0 |
| 9 | 0 | 0 | – | 0 | – | 2 | 0 | 0 |
| 10 | 0 | 0 | – | – | – | 3 | 0 | 0 |
| Total number of eggs laid from 2 to 7 day following insemination | | | | | | 25 | 8 | 6-day fertility rate 32.0 (8:25) |

decreased to 50 percent in both cases. The ratio of the number of fertile eggs to the total number of eggs produced during the period from the second to the seventh day following a single insemination of Common ducks with Muscovy semen was 32.0 percent (8:25), comparing with 38.6 percent (22:57) fertility of the homologous semen. Although the number of birds tested here is small, it is clear from this experiment that the repetition of every-three-day insemination will be very effective for retaining a satisfactory level of fertility higher than that obtainable in natural matings.

5. Hatchability, Pre- and Post-natal Development of Mule-Ducks

As for the hatching, 6 hybrid ducklings were secured from 8 fertile eggs showing 75 percent (6:8) hatchability and 25 percent (2:8) embryonic mortality. Although the two unhatched eggs had exhibited a vascular development at the first candling carried out on the fifth day of incubation, they showed no trace of embryonal tissues excepting the remains of the vascular system when they were examined by breaking their shells a week later. This suggests that the embryos had died in a very early stage of incubation, probably at the time of the first candling or therabout. The secondary sex ratio revealed 200 (♂♂ 4:♀♀ 2), the male being exceedingly predominating though the number of hatched eggs is not enough to give a decisive conclusion.

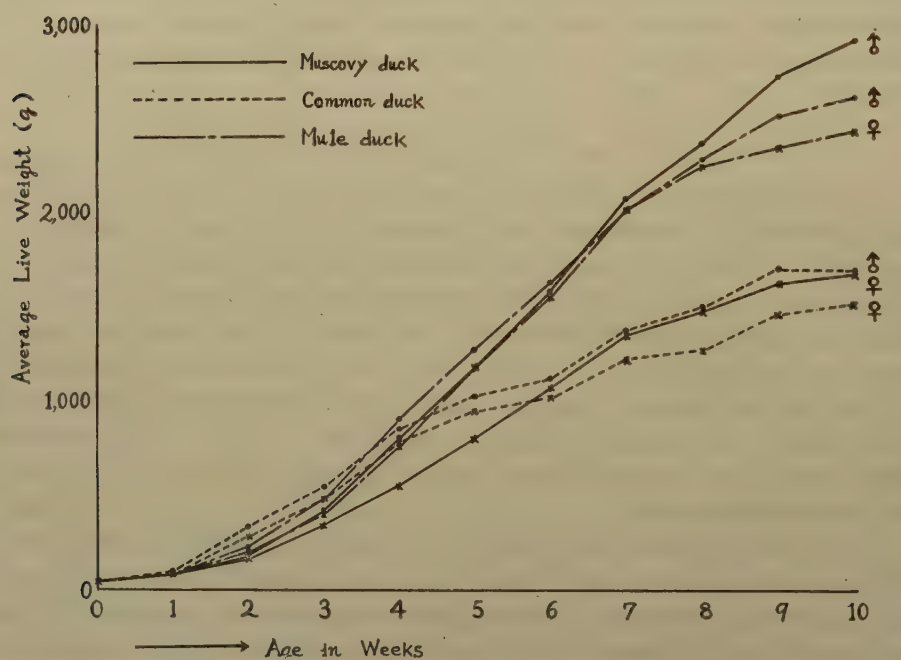
In the Common duck here observed, the length of incubation varied from 27 to 29 days with an average of 28 days, while that of the Muscovy varied from 35 to 37 days with an average of 36 days. Hybrid eggs occupied an intermediate position between those of the two parental species, ranging from 29 to 32 days with an average of 31 days in consistence with the finding of FABER (1957).

In live weight from hatching to the third week of age there were very little differences among the three breeds of ducks but with age they exhibited their own growth rates (Table XIV; Text-fig. 5).

Among them the Common duck grew most slowly, both the male and female showing almost the same growth rates. In 10 weeks after hatching, the male Com-

Table XIV. The average rates of growth of mule-ducks produced by insemination in comparing with those of their parent ducks. The figure in a parenthesis indicates the number of birds determined with regards their live weight (g).

| Age of ducklings | Common duck | | Muscovy duck | | Mule-duck | |
|---|---|---|---|---|---|---|
| | ♂(3) | ♀(14) | ♂(3) | ♀(1) | ♂(3) | ♀(1) |
| At hatching | 46 | 43 | 50 | 47 | 43 | 42 |
| 1 week after hatching | 99 | 84 | 81 | 78 | 85 | 82 |
| 2 weeks 〃 〃 | 331 | 280 | 182 | 164 | 232 | 202 |
| 3 〃 〃 〃 | 542 | 480 | 421 | 342 | 483 | 400 |
| 4 〃 〃 〃 | 847 | 782 | 803 | 550 | 905 | 761 |
| 5 〃 〃 〃 | 1,020 | 949 | 1,177 | 800 | 1,267 | 1,181 |
| 6 〃 〃 〃 | 1,121 | 1,023 | 1,579 | 1,065 | 1,628 | 1,551 |
| 7 〃 〃 〃 | 1,384 | 1,225 | 2,076 | 1,350 | 2,013 | 2,020 |
| 8 〃 〃 〃 | 1,505 | 1,277 | 2,372 | 1,480 | 2,293 | 2,250 |
| 9 〃 〃 〃 | 1,716 | 1,468 | 2,728 | 1,625 | 2,520 | 2,350 |
| 10 〃 〃 〃 | 1,703 | 1,526 | 2,920 | 1,680 | 2,618 | 2,440 |




Text-fig. 5. Graphs showing the growth rates of Common ducks, Muscovy and Mule-ducks.

mon duck weighed 1,703 g and the female, 1,526 g. On the contrary, the Muscovy duck exhibited a conspicuous sexual dimorphism in live weight; the male Muscovy showed the most rapid growth, whereas the growth rate of the female Muscovy did not vary greatly from that of the male Common duck. The live weight of the 10-week old male was 2,920 g and that of the female of the same age was 1,680 g. In this respect, the findings on the mule-duck were most remarkable; the male mule-duck had very high growth rate similar to that of the male Muscovy, attaining 2,618 g live weight at the age of 10 weeks and the female mule-duck of the same age also attained almost the same live weight as its male, that is, 2,440 g. Thus the mule-duck produced by artificial insemination shows the prominent character as a meat bird so well as that produced by natural mating.

## SUMMARY

1. An attempt was made to increase the fertility rate of hybrid eggs in mule-duck raising, applying the same technique of artificial insemination as used for the Common duck.

2. The semen of the Muscovy drake could be obtained by electrical stimulation with regular success, whereas the massage method was hardly applicable for this bird.

3. It was found that there is a vital difference in testicular activity between both the duck species, Muscovy and Common duck, the semen of the former showing fewer sperms both per cubic millimeter of semen and per ejaculate than that of the latter. The Muscovy semen, compared with that of the Common duck, is about 28 times smaller in the maximum sperm concentration and 23 times smaller in the maximum number of sperms of the monthly average (Tables III and XII).

4. Beside the difference in quantitative characters of the semen between the Muscovy and the Common duck, there exists a qualitative difference indicating lower physiological efficiency of sperms of the Muscovy.

5. In spite of lower fertility in the Muscovy, a single insemination with its semen alloted to five Common ducks showed 75 percent daily fertility per flock on the second day and 50 percent on the third and fourth day after the insemination. This suggests a high possibility 'of utilizing artificial insemination for mule-duck production, when it is performed every two or three days per bird.

6. Since the Muscovy semen in the present study was used for insemination without buffering but at relatively high dilution with the physiological saline solution, more satisfactory fertility level may be obtained, if the two points, a suitable buffer solution and a less dilution rate, will be further considered.

7. There was a tendency for prenatal disturbances which seemed to be caused by an intergeneric hybridization: A high embryonic mortality (25 percent) and a predomination of the male in the secondary sex ratio (=200) of the hybrid bird.

## VIII. DISCUSSION

The present paper dealing with the artificial insemination of ducks in connec-

tion with their reproductive physiology involves many problems hitherto obscure. Among them are some main problems taken in consideration for discussing in order.

1. Techniques of Semen Collection

Since the publication of classical work of IVANOV (1913), a number of methods of semen collection from the male bird have been reported. Aside from the post mortem collection by IVANOV, the techniques which are combined with the natural mating have many disadvantages in training the birds and in receiving semen without contamination or loss. In this impasse BURROWS and QUINN (1935, 1937) made the way of collecting semen, either applying abdominal massage or milking the copulatory organ of the male bird. In this respect KAMMAR (1958) has given a very useful review categorizing the techniques of collecting cook semen in a chronological order.

For the water-fowl, however, semen collection has been rarely reported and still now remains with a limited scope of experiments.

KUZIMINA (1933) was the first to report on the semen collection with success from drakes by applying a pessary to the cloaca of ducks. YAMASHINA (1950), ŌNISHI, FUTAMURA, KATO and AKAMATSU (1950) and ŌNISHI, KATO and FUTAMURA (1955) successfully applied massage method to domestic ducks, and likewise ROSE (1953), JOHNSON (1954) and KINNY and BURGER (1960), to geese. Another technique of semen collection an ejaculatory response by electrically stimulating the male bird. SEREBROVSKII and SOKOLOVSKAJA (1934) seemed to be the first authors who reported about the electrical stimulation applied to birds: 1 cock, 1 goose, 2 drakes, several pheasants and 13 guinea fowls. As the original paper of the authors was not accessible, a reference may be made here from an abstract in the Animal Breeding Abstract 3:73–74 (1935):

> "Ejaculation could be induced by an electric current, the positive pole being applied to the skin of the bird in the sacral region, the negative pole placed in a basin of water, into which the beak was immersed. As a rule a weak shock of 45 volts was given first, which caused spasms and sometimes ejaculation, but usually ejaculation was only produced by four stronger shocks (80 volts), each of 3–4 seconds' duration, with 1–2 seconds' intervals. Most of the experiments were successful, and normal sperm was regularly collected, though in small quantities. In several instances (e. g. some of the pheasants) failure was found to be due to sterility. No injurious influence was noticed and experimental casualities were few."

NISHIKAWA (1951) describes briefly his observations on the electrical stimulation of the fowl with alternating current at 40 volts applied per rectum and hypodermis of the sacral region in accordance with the method of GUNN (1936) devised for the ram. The author, however, is of opinion that the cock semen thus procured is too small in quantity to be used for practical purpose.

In this uncertain state of affairs, the present experiments were undertaken in using the two methods of semen collection: massage and electrical stimulation, with the aim of some technical improvements. Both the methods showed more or less advantages in practical use.

The massage method relying on the psychic response of the bird needs generally a long period of time for training the bird, commonly 10 to 15 days, which is

undoubtedly a laborious work for duck breeders. Besides, there are some birds which never respond to this operation. On the contrary, the electrical stimulation induces an ejaculation of semen quite reflexively regardless of preceding training or individuality of birds. No evil effect upon the operated bird is found and some skin injuries sporadically seen at massage can be avoided by this method. For semen qualities, too, the electrical method has a certain advantage as the semen is obtainable in less contaminated state. Hence, from the standpoint of operation, the electrical method is decidedly preferable to the massage. Two questions must, however, be raised before conclusion. One relates to the fertility and the second to the hereditary effect of the semen thus obtained.

Since the electro-ejaculation is an entire reflex without accompanying orgasm, the ejaculate thus secured is deficient in accessory sex secretions commonly known as seminal plasma. The sex secretions cannot be considered as ineffective for the viability and fertility of sperms. In the Common duck, the sperm concentration was found about 5 times higher and the total per-ejaculate sperm number 8 times greater in the case of electrical stimulation than in the case of massage. On the other hand, in the fertility tests with the semen likewise diluted ten times, the sperms from the electro-ejaculation showed clearly less daily fertility rates and a shorter duration of retaining fertility potential than those from the massage-ejaculation (Table VI, VII & VIII). In that condition, therefore, the semen collected by electrical stimulation is unlikely to be suitable for practical use.

There has been a generalized concept for mammals that the male accessory sex secretions cause a decreased viability and fertility of sperms (GUNN, 1936). The above data on the duck semen electrically collected are certainly in contradiction to this postulation. As a consequence, it seems to be more reasonable to attribute the low resultant fertility of the electro-ejaculated semen to the direct influence of electric current applied, possibly not only upon sperm cells but also upon their precursors during the spermatogenesis. In the present experiments, however, the electro-ejaculated semen, when it is diluted ten times with fowl egg-yolk citrate buffer, exhibited an average viability of $126 \pm 41.11$ hours with a range of 48–192 hours (Table IX) and a possibility of retaining 100 percent daily fertility for 4 days in association with 12 days of the maximum duration of fertility (Table X, Flock I). Hence, there is little reason to believe any possible effect of electric current applied and the electro-ejaculated semen can be used safely for practical purpose, if it is diluted with a suitable buffer.

More important is the problem of the effect of continuous electrical stimulation of drakes upon the offspring produced by insemination with their semen. As above mentioned, there has been no deleterious effect upon the parent birds operated but this does not always mean the absence of the indirect effect of electric current upon their offspring. With regard to the effect of electric stimulation on the chromosomes of germ cells the present knowledge is almost nil. Unless a comprehensive progeny test is made, no reliable conclusion will be gained, but it is outside the scope of the present experiments performed on a laboratory scale. At any

rate, there is nothing to worry about this, as far as it concerns the production of meat animals or animals of $F_1$ generation as mule-duck.

2. Techniques of Semen Introduction

Although application of a vaginal speculum for inducing the semen pipette is a usual technique for the insemination of mammals, no speculum has yet been used for birds. In the above experiments the convenient use of the speculum newly devised accommodating to duck's genital organs was demonstrated with regular success by a lot of fertility tests; it could be used with striking readiness neither giving any injury to birds nor reducing the fertility rate of eggs produced. It has so far been fully established that the use of speculum has the advantage over the palpation technique in the semen introduction to the oviduct.

As for the time of semen injection, there have been somewhat contradictory opinions for the fowl. It has been a generally accepted opinion that the presence of a hard-shelled egg in the uterus affects the fertility of forthcoming eggs, thence the insemination should be performed in the afternoon when the uterus is empty. The other opinion represented by SCHINDLER *et al.* (1957) is that no difference is found between the hens inseminated while containing a shell-egg in their uterus and those not containing such an egg with regard both to the fertility of hens and eggs laid.

In the duck there is as yet no criterion concerning the relation of the presence of a shell-egg to the fertility of forthcoming eggs. However, because of the observations that the semen was usefully obtained without being soiled early in the morning before supplying forages, and that the insemination could be performed more successfully with semen as fresh as possible, the forenoon insemination is more preferable for the duck. If the presence of shell-egg plays an inhibitory role in the fertilization of subsequently released eggs, the insemination in the forenoon should be decidedly preferable because ducks lay their eggs very early in the morning and the forenoon must be just the time when their uterus is empty.

3. Characteristics of Semen of Common Ducks

As regards the initial motility of sperms, the fresh, unsoiled ejaculate apparently shows the most satisfactory quality, regardless of methods of collection, about 80–90 percent sperms exhibiting very active movement producing strong currents in semen drops. The quantitative characteristics of semen, however, are remarkably different according to the techniques of collection. The sperm concentration of the semen electrically collected is 5 times higher and the semen volume is markedly smaller than the semen collected by massage; consequently the total per ejaculate number of sperms of the former is about 8 times as great as that of the latter. Besides, the interval of times of collecting semen, that is, the frequency of collection even by the same method, plays a great role in the variation of the numbers of sperms per cubic millimeter as well as per ejaculate. Generally speaking, a greater volume of semen is obtained after two- or three-day rest than after one-day rest within a limited period of time. As a rule, this is consistent with the findings of MUNRO (1938)

and BURROWS and TITUS (1939) in fowls, though the relationship between the frequency of collection and the volume of semen is no absolute one in the case of ducks; it was peculiar to observe that the drake could gradually adapt to the massage producing a greater volume of semen at an one-day rest than the volume of semen collected at a two- or three-day rest.

As one of the effective factors causing fluctuating in semen characteristics, not to be overlooked is the season, as has been reported on the fowl semen by several investigators: PARKER, McKENZIE and KEMPSTER (1942), PARKER and McSPADDEN (1943), WHEELER and ANDREWS (1943). In the duck, the total number of sperms per ejaculate is at its highest during the period from the middle of March to the beginning of July, rising to a peak in May, dropping to a bottom in the last half of August and recovering gradually from the end of September. Since the total number of sperms per ejaculate is regarded as a reflexion of degree of testicular activity, of the Common duck, this being consistent with the season of the highest production of eggs. On the contrary, the period from the fourth week of July to the third week of September appears to be the most unfavorable season for the breeding of Common ducks (Table III). This is also supported by the two experiments of insemination of 15 ducks at two different periods: the first half of September and of October; greater fertility rates and longer duration of retaining the fertilizing capacity of sperms were found in the experiment in October compared with the results in September.

Thus, the problem of semen characteristics seems to be too commplicated to get a generalized concept, because of too many factors involved, not to say the nutrition, leading to fluctuation both in quality and quantity of the semen.

4. Effects of Dilution Rates of Semen of Common Ducks upon Fertility

The practical importance of artificial insemination in poultry is to make the best use of the sire of top performance for breeding, by alloting a single ejaculate to as many dams as possible. On account of this, the problem of dilution of semen must be the first to take into consideration.

The effects of dilution of semen upon the viability and fertility of sperms are to be considered from the two aspects, provided that the dilutor itself is not harmful: One from the reduction of the effect of accessory sex secretions and the other from the reduction of sperm concentration.

It has been known in mammals that a ripe sperm stores a limited amount of energy, thence, whatever stimulates activity or the expenditure of that energy shortens its life, and that the violent activity displayed by sperms when dispersed in accessory secretions, is a main factor in shortening their length of life (HARTMAN, 1932; GUNN, 1936). There may be a similar phenomenon in birds, too.

In the present experiments, duck sperms just after collection exhibited always violent activity and retained a full fertility rate until the third day after insemination but a remarkable decrease in fertility was observed from later on. On the contrary, when the semen was diluted 5 times with physiological saline solution, it showed a full fertility rate until 7th day after insemination. This indicates clearly

a beneficial effect of dilution of the accessory secretions to a certain extent for the viability and fertility of sperms (Table IX). Since this was the result of a single insemination per bird, it follows naturally that a full fertility level will be maintained, if the insemination is carried out periodically with a cycle of six or seven days in the case of using the fresh semen (Table IX).

Considering that the physiological saline solution used for dilution contains no sources of energy for sperm activity, its beneficial action should be quite of physical nature and attributable, on one hand, to the reduction of the accessory secretions stimulating sperm activity, on the other, to the reduction of sperm concentration. The latter effect obviously relates to lowering the accumulation of metabolic products released from sperm cells. There is, however, a limit of beneficial effects of dilution because the experimental results showed that the time retaining a full fertility level was remarkably shortened when the semen was diluted 10 and 20 times. This concerns undoubtedly the excessive reduction of sperm concentration which naturally reduce the total number of sperms per fraction of semen to be injected.

According to PARKER, MCKENZIE and KEMPATER (1940) the fowl semen obtained by massage contains on the average 2,340,000 sperms per cu. mm. of semen and 868 millions per ejaculate. Using Ringer's solution, BONNIER and TRULSSON (1939) found that inseminations of hens with the semen of 3 times dilution gave better fertility than with pure semen and with the semen of 10-time dilution about the same fertility as with undiluted, but there was a decline in fertility from the semen diluted 50 times. ALLEN and SKALLER (1958) reported also that fowl semen, diluted 10 to 13 times with Tyrode's solution, when inseminated at the rate of 0.2 ml. twice weekly gave fertility results equal to those expected from undiluted semen or natural matings. Despite the fact that the duck semen contains a smaller number of sperms than that of the fowl, and that the dilutor used was deficient of nutritive substances, 10-time dilution of duck semen showed better fertility than in the fowl. This seems to indicate that duck sperms have a greater physiological efficiency than the fowl semen. There are, however, a considerable number of the authors who could not confirm such a beneficial effect of dilution: MUNRO (1938), GORDON and PHILLIPS (1951), SCHINDLER *et al.* (1955), ROWELL and COOPER (1957), and WILCOX (1958) for fowl semen, and GILBREATH and DAVIS (1949), Van TIENHOVEN and STEEL (1957), and SMYTH and KINNEY (1958) for turkey semen. This contradiction in view of the dilution of semen is very likely to be ascribed rather to the chemical properties of the dilutors which associated with the viability of sperms.

5. Effects of Chemical Composition of Dilutors upon Fertility of Semen of Common Ducks.

An analysis of the data (Table XI, Flock I) appears to indicate the full fertility of duck semen lasting to the fourth day after insemination when it was diluted 5 times with a fowl egg-yolk citrate buffer. Although the rates dropped on the second and fourth day, these can be assumed to fall into the period of retaining the full fertility potential. In contrast to this, for the semen diluted likewise 5 times with the physiological saline solution, the full fertility lasted until the sixth day after in-

semination (Table IX), showing two days longer duration of fertility potential. This difference in the effects of both the dilutors, the fowl egg-yolk citrate buffer and physiological saline solution, upon maintaining the fertility potential seems to show that the egg-yolk citrate buffer hitherto applied to the mammalian semen is not always the most suitable one for the duck semen.

Up-to-date, unlike mammals, the successful insemination of birds with stored semen is very little known, though the viability of stored sperms has been manifoldly reported. According to BURROWS and QUINN (1939) the cock semen stored both at 4.4°C and 20°C loses its fertilizing capacity within two and eight hours respectively. JASPER (1950) could obtain only one fertile egg by inseminating with cock semen that had been stored for 20 hours at 4.4°C in human serum, while GARREN and SHAFFNER (1952) secured 38 and 6 percent fertile eggs with undiluted cock semen stored at 5°C for 3 and 4 hours respectively. In the similar experiments of SCHINDLER *et al.* (1955) they found that the fowl semen diluted with whole milk and stored for 24 hours at 4°C gave only 19 percent resultant fertility though undiluted semen and the semen diluted with either Ringer's or Rock's solution or with whole milk, retained a full fertilizing capacity after 4 hour's storage at 10°C. The experiments of LAKE, SCHINDLER and WILCOX (1959) transporting fowl semen by air from U. S. A. to Israel and Scotland gave better results. The semen buffered with a phosphate solution containing antibiotics was cooled in an insulated container. The fertility rate of the semen thus transported to Israel wes 38 percent after 38 hours and that arrived in Scotland, 36 percent after 37 hours, computed from eggs laid within the first week following insemination. Quite recently LAKE (1960) reports the most promising results obtaining 64 and 47 percent fertility from the semen stored for 24 and 48 hours respectively at 0° to 2°C in a glutamate-containing saline solution added with fructose.

The present data on duck semen reveal also that, for the storage of semen, the more suitable buffers should be developed if its storage is necessary, although this appears to be of secondary importance in poultry husbandry.

6. Mule-Duck Production by Applying Artificial Insemination

In the present study, a striking performance of the mule-duck as a meat bird has been definitely shown (Table XIV and Text-fig. 5).

The male mule-duck gains almost the same live weight in 10 weeks as the male Muscovy. There is but little sexual dimorphism of live weight in the mule-duck, while this is much pronounced in the Muscovy. The Common duck shows only a little sexual difference in live weight but both the live weight and growth rate of this bird are small compared with those of the mule-duck. Sexual similarity in the live weight of the mule-duck is probably inherited from the mother bird, Common duck, and the big final body weight and the rapid growth rate may be due partly to the paternal inheritance and the heterosis.

The bottleneck in the mule-duck raising is, however, that the fertility of hybrid eggs is exceedingly low as has been above referred. This partial infertility of hybrid eggs has been ascribed by some duck-breeders in Taiwan to a difference in mating

behaviors between the parental species, the Muscovy being a land-copulatory bird and the Common duck, a water-copulatory bird. In the present study, observations of natural matings between the Muscovy drake and the Common duck, has revealed that the copulation is actually impeded by the difference in body weight between both the duck mates, the adult Muscovy drake being 2.3 times as heavy as that of the adult Common ducks. In spite of the vigorous sexual activity of the Muscovy drake and the receptivity of the Common duck, the latter can hardly bear the weight of the former riding on her back. It appeared that the conditions should be different if the copulation happened floating on the water, because this is usual habit of the Common duck. Nevertheless, no case was met with where the copulation was interrupted without showing ejaculatory reflex. It is therefore not likely that the partial infertility of hybrid eggs is caused by the physical hindrance in natural matings of the two duck species. The main reason should be rather sought in the characteristics of Muscovy semen (WATANABE, 1959).

The Muscovy semen, compared with that of the Common duck, is about 28 times smaller in the maximum sperm concentration and 23 times smaller in the peak number of sperms of the monthly average (Table III and XII). Therefore, it may well be doubted that the inseminations of Common ducks with the Muscovy semen diluted 10 times will result in lower fertility rate than in the case of inseminations with the Common duck semen of the same dilution rate. As a matter of fact, it appeared a marked difference in the weekly fertility, revealing 32.0 percent (8:25) of the Muscovy semen and 42.2 percent (19:45) of the Common duck semen, notwithstanding that the semen of both ducks were collected in the same manner by electrical stimulation (Tables VII and XIII). A further analysis of the daily fertility rates resulting from the inseminations of Common ducks with the homologous and heterologous semen, seems to indicate less physiological efficiency of the sperms of the Muscovy duck compared with the semen of the Common duck. There is a tendency of losing the fertilizing capacity earlier of the Muscovy sperms than those of the Common duck.

In the literature of duck breeding, YAMASHINA (1950) was the first worker who attempted to apply the artificial insemination to the mule-duck production; he secured 6 birds out of 14 intergeneric eggs produced by mating the female Muscovy ducks with a male Common duck, showing a fertility of 42.8 percent. However, a reciprocal cross, namely a cross between the Common ducks with a Muscovy drake brought no satisfactory results in fertility. The cause of this failure was ascribed by the author to the sterility of the male Muscovy employed, because the semen was found to be devoid of sperm cells. Later, ŌNISHI & KATO (1955) succeeded in obtaining fertile eggs by artificial insemination of Common ducks with Muscovy semen. However, no marked increase in the fertility rate of hybrid eggs was attained by inseminating beyond that secured by natural matings, the rate of the former being 34.2 percent (26:76) and that of the latter 32.0 percent (96:300). Thus, the fertility rate of hybrid eggs, whether artificially inseminated or naturally mated, was lower than that of Common duck's eggs procured by the homo-insemination in

another experiment (ŌNISHI, KATO & FUTAMURA, 1955); in the latter experiments the fertility rate was 54.2 percent (130 fertile eggs to 240 produced).

It is known that the Common duck in its domesticated state is polygamous and exhibits a high testicular activity almost all the year round excepting a few months, whereas its wild ancestor, the Mallard, is monogamous and breeds only in a certain season. A conversion of the Mallard from monogamous bird into polygamous bird and an aquisition of a habit of breeding all through the year is regarded as the effect of constant selection with respect to its high fecundity, to which this bird has been subjected from time immemorial. In contrast to this, the Muscovy duck of South American origin, though domesticated prior to importation to Europe, seems to have been very little selected with respect to its fecundity. This is to be seen in the lower yield of sperms throughout the year in the Muscovy. It seems, therefore, to be fair to conclude that less resultant fertility rate of the semen of Muscovy is a reflexion of less selection of this bird in respect to fecundity. As for the lower physiological efficiency of Muscovy sperms manifested in the daily fertility rate, it is not certain whether this is of genetical nature or of environmental influence caused by the genital tracts of Common ducks or of both effects in association.

Although the present data applying the artificial insemination for the production of mule-ducks has shown low fertility rate of hybrid eggs, it is not discouraging, if considered, that despite a single insemination made per bird during an unfavorable breeding season (the 4th week, September), the daily fertility was 75 and 50 percent on the second and third and fourth day after insemination respectively. If the insemination had been done every two or three days per bird, the fairly satisfactory fertility level would have been continuously retained. And there is every reason to believe that the fertility rate can be promoted further by using a more suitable dilutor and diluting to a smaller degree than that used here.

From this point of view, it may fairly be said that the development of the mule-duck industry is now within reach by the use of the techniques of artificial insemination described above.

## IX. CONCLUSION

Recent advances in the artificial insemination of farm animals have amazingly contributed to the revolutionary development of animal industry.

In the poultry husbandry, however, the situation has been so far quite otherwise; the application of artificial insemination to the poultry has been quite handicapped until BURROWS and QUINN (1935) reported a technique of collection of cock semen. This seems partly to be due to less understanding of the economic importance of artificial insemination for the poultry industry, associated with the technical difficulties of semen collection in birds.

Especially for the duck, no attempt has so far been made for applying the artificial insemination to the practical breeding to any great extent.

In the *status quo*, the results of the foregoing experiments of artificial insemination with ducks justify the conclusion that the techniques there described in collect-

ing and introducing the semen can be put into practice with great ease. The use of an electro-ejaculator for collecting the semen and the use of a speculum newly devised for inseminating have exceedingly simplified the whole procedure for routine insemination purposes. For the Common duck, it has been shown that a full fertility level can be retained by a periodical insemination with a cycle of five days in the case of immediate use of semen after collecting and of three days in the case of 24- or 48-hour storage of it. As regards the promotion of the fertility rate of hybrid eggs in mule-duck production, the results were not so satisfactory as in the Common duck with homologous semen in spite of the same technique applied. This should be partly attributed to the intergeneric crossing of both the birds remotely related and also to the lower spermatogenic activity of the Muscovy than that of the Common duck. Nevertheless, the application of the above techniques resulted in higher fertility rate of hybrid eggs than by natural matings; it suggests also, that this fertility level will be retained, if the insemination will be done periodically every two or three days per bird.

Technique is still being improved, especially for preparing the dilutor most suitable for the semen of duck species. So one may conclude that there is the commercial possibility of setting up the hatcheries applying the artificial insemination on an extensive scale and producing the largest possible number of ducklings. Thereby, a considerable expansion of duck industry is expected in the rice-cultivating countries of Asia, where meat and eggs of this bird are enormously demanded and the great potentialities of forage supply and pasturing are existing.

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## EXPLANATION OF PLATES I–IV

### Plate I.

Fig. 1. The pose of a drake fixed on a holder before being massaged; the side-plate is removed.
Fig. 2. Receiving semen into a tube.
Fig. 3. Receiving semen from the spirally evaginated penis following massage. The spiral ejaculatory groove is seen on the penis.
Fig. 4. The same as Fig. 3.

### Plate II.

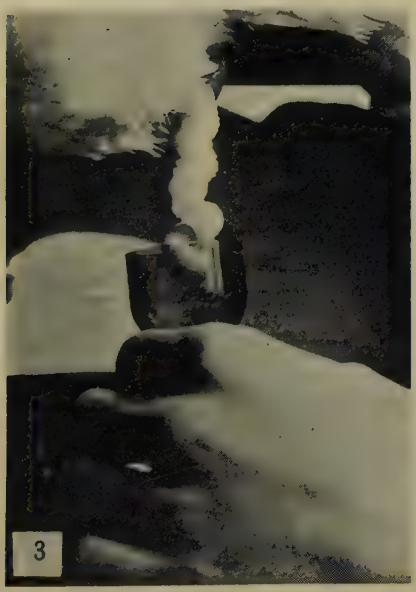
Fig. 5. An electro-ejaculator constructed by FHK Co. Ltd., Tokyo; the apparatus was applied to drakes in the present experiment.
Fig. 6. Two electric poles of the electro-ejaculator: the sharp one for sticking to the hypodermis of the ilial region and the blunt rod for inserting into the vent.
Fig. 7. A drake prepared for turning the current, the two poles being attached.
Fig. 8. At the moment of turning the current; the bird shows the rigidity of its body.
Fig. 9. Pressing of the stiff penis with fingers.
Fig. 10. Collection of ejaculated semen into a receptacle.

### Plate III.

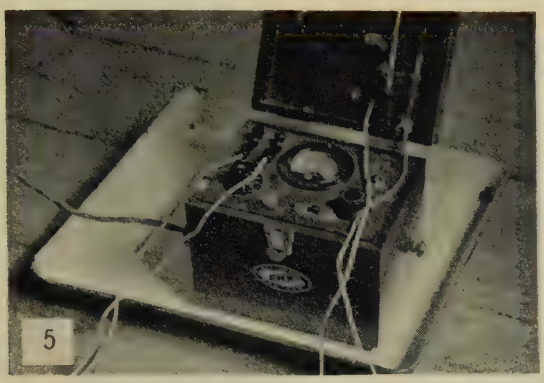
Fig. 11. A vaginal speculum devised for ducks; a side view.
Fig. 12. The same as Fig. 11; upper view.
Fig. 13. Equipments for semen injection: semen vials, speculum and insemination pipette.
Fig. 14. Orienting the vaginal orifice in the vent.
Fig. 15. Insertion of the speculum through the vaginal orifice.
Fig. 16. Insertion of an insemination pipette along the speculum.

### Plate IV.

Fig. 17. A Muscovy drake used for collection of semen; about 2 years old.
Fig. 18. A Common duck inseminated with the Muscovy semen; about 1 year old.
Fig. 19. A male mule-duck produced by the insemination; about 14 weeks old.
Fig. 20. A female mule-duck produced by the insemination; about 14 weeks old.



WATANABE : Artificial Insemination of Domestic Ducks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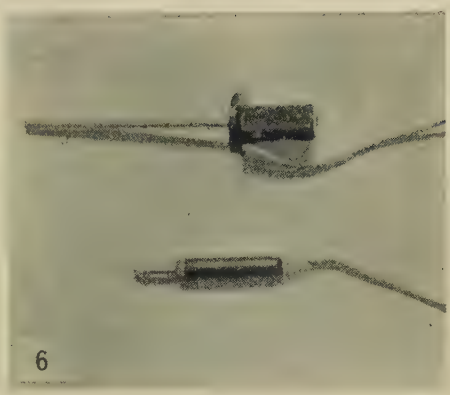
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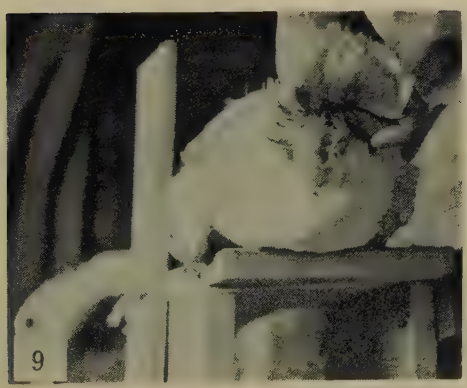
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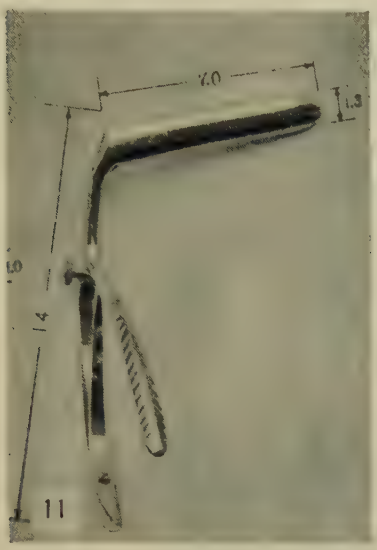
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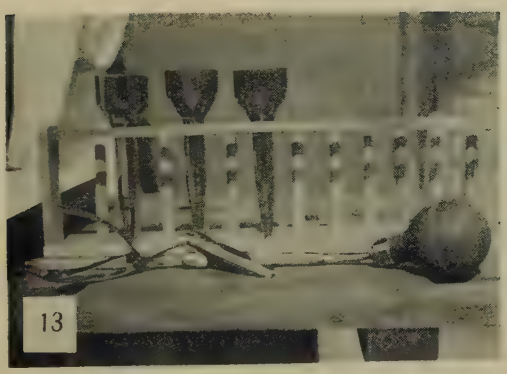
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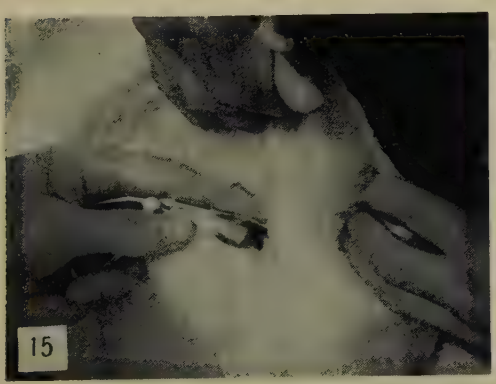
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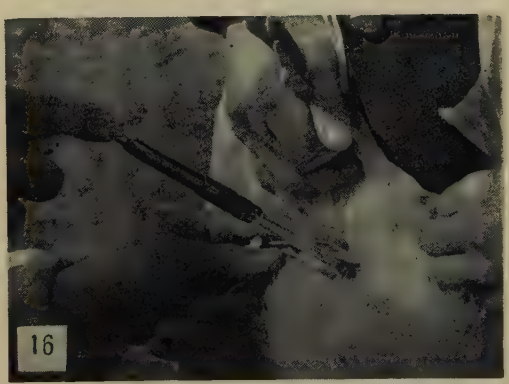
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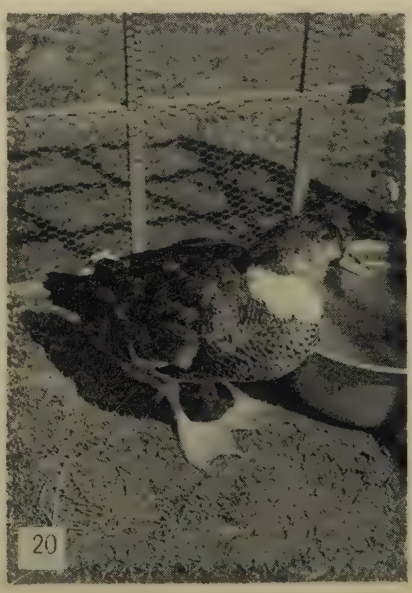
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# Studien über Zitronensäure in Kuhmilch

## I. Jahreszeitliche Schwankungen des Gehaltes an Zitronensäure der Kuhmilch

Yûzô Anagama und Takayasu Kami

*Institut für Tierzuchtlehre der Fakultät für Fischerei- und Tierzuchtlehre an der Universität Hiroshima, Fukuyama, Japan*

(Tabellen 1~3)

### (I) EINLEITUNG

Es ist erkannt worden, dass Zitronensäure in Kuhmilch bei Bildung des Butteraromas[1~12], bei Besserung der Käsequalität[13~17], bei Hitzestabilisierung der Kuhmilch[18~24], bei Stabilisierung tiefgefrorener Milch[25] und bei der Utrecht-Abnormalität der Milch[26][27], bei all diesen Vorgängen eine Rolle spielt und als Antioxydantien in Milch und Milchprodukten[28~30] wirksam ist. Aber die Faktoren, welche den Zitronensäuregehalt der Kuhmilch beeinflussen, sind noch nicht genau erklärt worden.

Über den jahreszeitlichen Einfluss auf die Höhe des Gehaltes an Zitronensäure gehen die Meinungen der verschiedenen Autoren bis in die jüngste Zeit auseinander. Hess *et al.*[31] sowie Supplee & Bellis[32] in USA fanden, dass der Zitronensäuregehalt der Kuhmilch kleiner in Stallfütterungsperiode als in Weidefütterungsperiode war. Dagegen berichteten Catalano[33] in Italien sowie Reinart & Nesbitt[34] in Manitoba in Kanada, dass der Zitronensäuregehalt der Kuhmilch grösser in Winter als in Sommer war.

Nach Sherwood & Hammer[35] sowie Arup[36] in USA waren die jahreszeitlichen Schwankungen des Zitronensäuregehaltes der Kuhmilch nicht signifikant.

Holwerda[37] untersuchte den Einfluss der Jahreszeit auf den Zitronensäuregehalt der Anlieferungsmilch in Friesland in Holland und fand niedrige Werte in den Monaten November, Dezember und Januar (Minimum in Januar). Im Februar und März lagen sie etwas höher. Es wurde jedoch vermutet, dass die Laktationszeit einen Einfluss hat, da in diesem Distrikt in den Monaten November bis Januar viele Kühe am Ende der Laktation stehen, während im Februar und März neue Laktationen beginnen.

Neuerlich bestimmte Nickerson[38] in 258 Sammelmilchproben aus verschiedenen Gebieten der USA 23 Milchbestandteile und fand, dass die jahreszeitlichen Schwankungen bei Zitronensäure signifikant waren.

Die vorliegende Arbeit berichtet über die Ergebnisse der Untersuchung dieses Problems unter dem Klima in Westjapan.

## (II) MATERIAL UND METHODE

Die Untersuchung wurde ausgeführt an gemischten Milchproben (von Holsteinrasse), welche aus den 3 Molkereien der Stadt Fukuyama in Hiroshima-ken in je 5 Tagen von jeder Molkerei monatlich während eines Jahres gewonnen wurden.

Die Untersuchung wurde in der Zeit von Mitte Mai 1960 bis Ende April 1961 durchgeführt.

Der Zitronensäuregehalt der Kuhmilch wurde nach MARIER & BOULET[39]~[42] bestimmt.

## (III) ERGEBNISSE UND DISKUSSION

Tabelle 1. Zitronensäuregehalt der Kuhmilch im Verlaufe des Jahres (mg/100 ml)

| Monat \ Probe Nr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Mai '60 | 219 | 214 | 214 | 211 | 165 | 190 | 210 | 202 |
| Juni | 227 | 224 | 225 | 210 | 211 | 212 | 227 | 220 |
| Juli | 156 | 135 | 158 | 144 | 161 | 159 | 158 | 145 |
| Aug. | 140 | 159 | 150 | 127 | 136 | 144 | 112 | 116 |
| Sep. | 159 | 192 | 176 | 159 | 175 | 144 | 156 | 154 |
| Okt. | 188 | 193 | 196 | 185 | 192 | 192 | 193 | 187 |
| Nov. | 216 | 192 | 201 | 199 | 217 | 197 | 218 | 220 |
| Dez. | 221 | 223 | 221 | 219 | 214 | 239 | 215 | 201 |
| Jan. '61 | 220 | 212 | 215 | 211 | 214 | 225 | 221 | 234 |
| Feb. | 218 | 211 | 215 | 211 | 212 | 211 | 215 | 219 |
| März | 218 | 218 | 215 | 220 | 214 | 216 | 224 | 219 |
| Apr. | 212 | 205 | 206 | 208 | 214 | 199 | 181 | 184 |

| Monat \ Probe Nr. | 9 | 10 | 11 | 12 | 13 | 14 | 15 | **Mittel** |
|---|---|---|---|---|---|---|---|---|
| Mai '60 | 208 | 197 | 207 | 197 | 197 | 203 | 210 | **203** |
| Juni | 221 | 196 | 219 | 161 | 192 | 197 | 165 | **207** |
| Juli | 118 | 122 | 125 | 122 | 129 | 113 | 103 | **137** |
| Aug. | 158 | 125 | 141 | 123 | 147 | 117 | 119 | **134** |
| Sep. | 131 | 175 | 119 | 170 | 156 | 174 | 172 | **161** |
| Okt. | 172 | 180 | 168 | 175 | 201 | 172 | 158 | **183** |
| Nov. | 225 | 223 | 202 | 214 | 223 | 221 | 216 | **212** |
| Dez. | 201 | 208 | 215 | 202 | 220 | 214 | 223 | **216** |
| Jan. '61 | 215 | 223 | 211 | 208 | 210 | 215 | 215 | **217** |
| Feb. | 230 | 185 | 219 | 218 | 216 | 214 | 215 | **214** |
| März | 230 | 241 | 220 | 215 | 215 | 229 | 224 | **221** |
| Apr. | 185 | 203 | 207 | 206 | 210 | 212 | 210 | **203** |

Tabelle 1 zeigt, dass der Zitronensäuregehalt der Kuhmilch im März mit 221 mg/100 ml am höchsten und im August mit 134 mg/100 ml am niedrigsten ist. Der Zitronensäuregehalt der Kuhmilch im März ist im Durchschnitt beinahe gleich dem durchschnittlichen Gehalt im Dezember oder Januar, aber er ist signifikant höher

als die durchschnittlichen Gehalte anderer Kalendermonate. Und der Zitronensäuregehalt der Kuhmilch im August ist im Durchschnitt beinahe gleich dem durchschnittlichen Gehalt im Juli, aber er ist signifikant niedriger als die durchschnittlichen Gehalte anderer Kalendermonate. Dieses Untersuchungsergebnis, dass der Zitronensäuregehalt der Kuhmilch grösser im Winter als im Sommer ist, stimmit denjenigen von CATALANO[33] in Italien und von REINART & NESBITT[34] in Kanada überein.

Worauf ist es nun zurückzuführen, dass der Zitronensäuregehalt der Kuhmilch im Sommer sich vermindert? Wir[43]~[45] fanden, dass die Erniedrigung des Gehaltes an fettfreier Trockenmasse der Kuhmilch im Sommer im Fukuyama-Distrikt hauptsächlich durch Verminderung des Kaseingehaltes verursacht wurde, wobei auch die Senkung des Milchzucker- und Molkeneiweissgehaltes eine Kleine Rolle spielte, und dass es vielleicht hauptsächlich auf die hohe Lufttemperatur mit hoher Luftfeuchtigkeit des Sommers in diesem Distrikt zurückzuführen sein wird. In neuerer Zeit berichteten KAMAL *et al.*[46], dass die hohe Lufttemperatur über 80°F (ca. 27°C) die Zitronensäure in der Kuhmilch verringerte.

Es wird vielleicht hauptsächlich auf die hohe Lufttemperatur mit hoher Luftfeuchtigkeit des Sommers in diesem Distrikt zurüchzuführen sein, dass in diesem Falle der Zitronensäuregehalt der Kuhmilch im Sommer sich verminderte (vgl. Tabelle 2).

Tabelle 2. Lufttemperatur und Luftfeuchtigkeit im Fukuyama-Distrikt

| Monat | Mai 1960 | Juni | Juli | Aug. | Sep. | Okt. | Nov. | Dez. | Jan. 1961 | Feb. | März | Apr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lufttemperatur (°C) | | | | | | | | | | | | |
| Min. | 5.2 | 11.3 | 20.9 | 18.3 | 11.7 | 4.9 | 0 | −4.4 | −5.1 | −5.3 | −4.5 | −1.6 |
| Max. | 30.2 | 32.3 | 35.2 | 36.4 | 31.2 | 26.5 | 22.1 | 16.6 | 11.3 | 12.9 | 19.0 | 22.9 |
| Mittel | 17.6 | 21.5 | 27.0 | 27.8 | 23.3 | 16.6 | 12.1 | 5.4 | 2.5 | 3.5 | 8.1 | 13.4 |
| Luftfeuchtigkeit (%) | | | | | | | | | | | | |
| Mittel | 75 | 78 | 81 | 76 | 85 | 75 | 76 | 71 | 70 | 66 | 70 | 69 |

(Nach den Messungen der Wetterwarte Matsunaga)

Aus Tabelle 1 ist es auch ersichtlich, dass der Zitronensäuregehalt der 180 Sammelmilchproben (von Holsteinrasse) 103 mg als Minimum, 241 mg als Maximum und 192 mg im Durchschnitt in 100 ml betrug (= ca. 0.103~0.241%, im Mittel 0.192%). Die bisher von anderen Autoren berichteten Untersuchungsergebnisse über den Zitronensäuregehalt der Kuhmilch sind zum Vergleich in Tabelle 3 zusammengestellt.

Danach schwankt der Zitronensäuregehalt der Kuhmilch zwischen 0.07% (SHERWOOD & HAMMER[35]) und 0.40% (KIEFERLE *et al.*[48]). Fasst man alle bisherigen Untersuchungsergebnisse zusammen, so ergibt sich ein mittlerer Gehalt von ca. 0.18%. In den neueren Handbüchern ist der Zitronensäuregehalt der Kuhmilch folgenderweise angegeben: von ROEDER[64] 0.2~0.25%, von DAVIS[65] 0.2%, von LING[66] 0.18%, von FRANDSEN[67] 0.15~0.30%, von BRUNCKE[68] 0.24~0.26%

und von JENNESS & PATTON[69] 0.2%.

Tabelle 3. Zitronensäuregehalt der Kuhmilch

| Autoren | Jahr | % oder g/100 ml |
|---|---|---|
| SOMMER & HART[47] | 1918 | 0. 191～0. 248% (Im Mittel 0. 231%) |
| HESS *et al.*[31] | 1920 | 0. 08% (Stallfütterung)<br>0. 13% (Weidefütterung) |
| SUPPLEE & BELLIS[32] | 1921 | 0. 142% (Stallfütterung)<br>0. 148% (Weidefütterung) |
| KIEFERLE *et al.*[18] | 1925 | 0. 2～0. 4% |
| SHERWOOD & HAMMER[35] | 1926 | 0. 07～0. 33% (Im Mittel 0. 18%) |
| HOLM *et al.*[23] | 1932 | 0. 19～0. 29% |
| SATO & MURATA[49] | 〃 | 0. 096% |
| HARTMAN & HILLIG[50] | 1933 | 0. 14～0. 19% (Im Mittel 0. 16%) |
| ARUP[36] | 1938 | 0. 150～0. 206% |
| HEINEMANN[51] | 1944 | 0. 170～0. 201% (Im Mittel 0. 185%) |
| OVERBY[52] | 1945 | 0. 17% (Gewöhnliche Milch)<br>0. 21% (Jerseymilch) |
| STORGÅRDS[53] | 1948 | 0. 122～0. 258% (Im Mittel 0. 18%) |
| FABRIS[54] | 1951 | 0. 215～0. 290% (Im Mittel 0. 254%) |
| BABAD & SHTRIKMAN[55] | 〃 | 0. 180～0. 245% (Im Mittel 0. 193%) |
| EL-NEGOUMY[56] | 1954 | 0. 148～0. 200% |
| HOLWERDA[37] | 〃 | 0. 131g/100ml (Januar)<br>0. 173g/100ml (April) |
| ZIEGLER[57] | 1956 | 0. 090～0. 270% |
| CATALANO[33] | 〃 | 0. 217% (Wintermilch)<br>0. 200% (Sommermilch) |
| GOBIS & TAGLIABURE[58] | 1957 | 0. 200g/100ml |
| VERMA & SOMMER[59] | 〃 | 0. 157g/100ml |
| BALBA *et al.*[60] | 1958 | 0. 183g/100ml |
| BUDARINA[61] | 〃 | 0. 17～0. 20% |
| WHITE & DAVIES[62] | 〃 | 0. 166～0. 192% (Im Mittel 0. 176%) |
| REINART & NESBITT[34] | 1959 | 0. 159～0. 186% (Im Mittel 0. 172%, Sammelmilch)<br>0. 142～0. 186% (Im Mittel 0. 168%, Herdenmilch) |
| PIJANOWSKI & DLUZEWSKI[63] | 〃 | 0. 141% (Kühe mit niedrigerer Milchleistung)<br>0. 124% (Kühe mit höherer Milchleistung) |
| NAKANISHI & TOKITA[17] | 〃 | 0. 083～0. 196% (Januar)<br>0. 152～0. 264% (Juni) |
| NICKERSON[38] | 1960 | 0. 154% (Februar～April)<br>0. 157% (Mai～Juli)<br>0. 146% (August～Oktober)<br>0. 142% (November～Januar) |

## (IV) ZUSAMMENFASSUNG

(1) Der Zitronensäuregehalt der Kuhmilch im Fukuyama-Distrikt (Westjapan) wurde während eines Jahres bestimmt.

(2) Der Zitronensäuregehalt der Kuhmilch war im März mit 221 mg/100 ml am höchsten und im August mit 134 mg/100 ml am niedrigsten. Es ist zu vermuten, dass die Erniedrigung des Gehaltes an Zitronensäure der Kuhmilch im Sommer

vielleicht auf die hohe Lufttemperatur mit hoher Luftfeuchtigkeit des Sommers in diesem Distrikt zurückzuführen sein wird.

(3) Der Zitronensäuregehalt der 180 Sammelmilchproben (von Holsteinrasse) betrug 103 mg als Minimum, 241 mg als Maximum und 192 mg im Durchschnitt in 100 ml.

Wir danken herzlich Herrn H. HECKER für seine sehr freundliche und sorgfältige Berichtigung des Manuskriptes.

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# Light-Microscopic Studies on Changes in the Ultrastructural Density of Skeletal Muscle Fibers Caused by Thermal Treatment

Shunsaku Fujii

*Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Text-figs. 1–5)

Thermal treatment, such as boiling and freezing, of muscle is applied not only occasionally to histological studies, but also widely to the preservation of meat. It is well known that thermal process brings about pronounced morphological changes to the structure of muscle. Many histological studies have been made on injured muscle. For instance, Sakuma (1924), Häggqvist (1932), and Abe (1932) have observed changes in boiled muscle and Plank (1916) and Chamber (1932) those in frozen muscle. In addition to the structural changes, considerable changes in the ultrastructure of muscle fiber must be involved in the thermal process, since the process causes such definite alternations in the colloidal system of the muscle cell as coagulation, swelling, and disintegration of cell elements. No observation, however, has been made on these ultrastructural changes.

Seki (1954) stated that the ultrastructural density of cell elements could be determined by examining stained specimens under the light microscope. The theory and technique of such method were elaborated by Möllendorff (1924) and Seki (1936, 1954). The principle of the theory is explained as follows: The ultrastructural density of cell elements is estimated from the intensity of the color tone of a specimen stained with a combination of two or more acid dyes which differ in dispersity and color tone. For example, the azan method, recommended by Seki for this purpose employs three acid dyes, orange G, azocarmine B, aniline blue. The cell elements present densely in the ultrastructure are stained with small-moleculed orange G in a yellow tone. Those present less densely are stained with moderate-moleculed azocarmine in a red tone. Those present coarsely are stained with large-moleculed aniline blue in a blue tone. In fact, the cell elements stained with azan are presented in various colors accompanied by a different degree of ultrastructural density.

The present investigation was performed, on the basis of above-metioned theory, to observe ultrastructural changes in skeletal muscle which was evoked by the thermal process, using several muscles of different animals. Comparison was also made between different methods or temperatures of boiling and freezing and the resulting changes in different muscles.

## (I) MATERIALS and METHODS

The muscles used were obtained from healthy adult animals just after killing.

They consisted of the longissimus dorsi muscle of a cow and the longissimus dorsi (white muscle) and the masseter (red muscle) muscle of a rabbit as mammals, the pectoral (white muscle) and the quadriceps femoris muscle of a hen as bird, and the dorsal muscles of a golbefish, *Spheroides rubripes,* and a sciainoid fish, *Pseudosciaena undovittata,* as fish. The muscles were cut into blocks of the same shape and $1 \times 1 \times 0.5$ cm in size. Thermal treatment was performed in two methods, boiling and freezing. In boiling, material was heated for ten minutes in a water bath at 30, 40, 50, 60, 70, or 100°C, the temperature being maintained exactly during the treatment. Then, it was fixed with 10% formalin. Two methods of freezing, a slow and rapid, were employed. In one method, material was frozen slowly in a refrigerator at about −10°C for two hours. In the other method, material was frozen rapidly in one minute with a freezing microtome. Frozen specimens were put directly in 10% formalin to avoid a removal of drip at thawing. Fixed specimens were imbedded in paraffin, sectioned at 7$\mu$ in thickness, and mounted on slides. The azan method of Heidenhain was employed to examine the ultrastructure of the cell. Hematoxylin and eosin and Heidenhain's iron hematoxylin were used for the observation of the cell structure.

Sections to be compared were cut as equally thick as possible, mounted on the same slide, and stained at the same time, so that experiments might be conducted under the same conditions.

## (II) RESULTS and DISCUSSION

1. Observation on boiled muscle.

a. Muscle of cattle.

In unboiled control muscle, muscle fibers were presented in a red to slight yellowish-red tint with azan. Myofibrils and cross striations of muscle fibers were poorly differentiated.

In muscle boiled at 30°C (30°–muscle for short), muscle fibers were all of the same stained color and structural aspect as those before boiled. In other words, the ultrastructure of muscle fiber was not yet changed in boiling at this temperature.

In 40°–muscle, muscle fibers were stained red to purplish-red with an increased blue tone, as compared with 30°–muscle. Such an increase in blue tone indicates that ultrastructural changes have occurred in fibers themselves. In this case, it is assumed that the ultrastructure of muscle fiber has become loose because of an increase in affinity for the large-moleculed aniline blue. The appearance of fibrils and cross-striations was more distinct than 30°–muscle.

In 50°–muscle, muscle fibers were stained purple to purplish-blue with a distinct increase in blue tone and fibrils, as well as cross-striations, were more distinguishable than 40°–muscle. From a marked increase in blue tone, it is surmised that the ultrastructure of muscle fiber has become loose to a large extent. In addition to these ultrastructural changes, microscopic structural changes, such as a partial collapse of myofibrils, took place in boiling at this temperature.

In 60°–muscle, muscle fibers were stained blue entirely. Cross-striations were noticeable in a slightly bending form, although fibrils became almost indistinct. Detailed observation disclosed that sarcolemma had been separated from the cell body due to the shrinkage of sarcoplasm and that the resulting space under the sarcolemma was filled with a granular substance which was derived from the protoplasm of muscle fibers. Intracellular connective-tissue fibers underwent degeneration to form a netlike structure. It must be noted that the ultrastructural density of muscle fiber became the lowest in boiling at this temperature, which nearly coincided with the temperature at which muscle proteins coagulated.

In 70°–muscle, muscle fibers were stained purplish-blue in a more decreased blue tone than in 60°–muscle. Decomposition of fibers was very conspicous. In short, 70°–muscle was not so coarse in ultrastructure as 60°–muscle, because that showed a more decrease in blue tone than this.

In 100°–muscle, muscle fibers were stained purple to reddish-purple in an increased red tone. Muscle fibers were apparently structureless, as a whole, owing to the disappearance of transverse and longitudinal striations of muscle fibers. The endomysial space was occupied by a large amount of disintegrated substance from myofibrils. Therefore, the ultrastructural density of 100°–muscle was higher than that of 70°–muscle, but was much lower than that of unboiled muscle. It should be noted that the muscle boiled at a temperature higher than 70°C was, in spite of spectacular damage in its structure, not so coarse in ultrastructure as that boiled at 60°C.

Generally speaking, the ultrastructural density of muscle fiber of cattle was not changed by boiling at a temperature below 30°C. It became a little lower by boiling at 40°C and much lower at 50°C, being the lowest after boiling at 60°C. After that, it showed opposite gradients of change with elevation of boiling temperature. From these results, it can be said that boiling process has an effect of making the ultrastructure of muscle fiber coarse.

As is well known, the principal constituent of muscle fiber is muscle proteins. They are composed of myogen, the globulin complex, coagulating at 50 to 60°C and myosin, the albumin complex, coagulating at 45 to 50°C. The principal factor which induces coarseness in the ultrastructure of muscle fiber may be considered coagulation of muscle proteins and other constituents. This assumption has been made from the fact stated previously that the temperature at which the ultrastructure of muscle fiber becomes the coarsest corresponds to the coagulation temperature of the above-mentioned muscle proteins.

In fact, when coagulation takes place among the cell elements of muscle fiber, including muscle proteins, and partial dissolution or swelling occurs as a result of boiling, the crevices among molecules or micelles will be enlarged. Accordingly, the ultrastructural density of the cell elements becomes low. So long as boiling temperature is high, however, coagulation is accelerated with a rise in temperature and dissolution or effusion is checked in these cell substances. So the crevices among molecules will not be enlarged so well as in the preceding case. Therefore, the ultra-

structure of the cell elements becomes denser than before. This assumption is supported by the fact mentioned previously that muscle boiled at 100°C had a higher ultrastructural density than that at 60°C.

b. Muscle of rabbit.

Rabbit muscle underwent the same changes in ultrastructural density as cattle muscle when boiled.

c. Muscle of chicken.

Chicken muscle showed a different relationship between boiling temperature and changes in ultrastructural density from that exhibited by cattle muscle. When stained with azan, chicken muscle revealed the following relationships between the color and boiling temperature: yellowish-red in unboiled state, purplish-red at 40°C, purple at 50°C, purplish-blue at 60°C, and purple at 70 and 100°C. These relationships indicate that the gradient of change in ultrastructure was essentially the same in chicken muscle as in cattle muscle. In the stainability of muscle fiber in a blue tone, chicken muscle was a little inferior to cattle muscle.

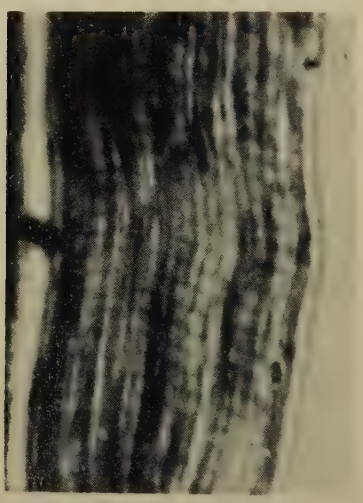

Fig. 1. Quadriceps femoris muscle of hen unboiled. Longitudinal section. Heidenhain's iron-hematoxylin staining. Myofibrils and cross-striations are poorly visible, being in a contruction stage with A disks short and I disks tall in height. ×1500.

Fig. 2. Quadriceps femoris muscle of hen boiled at 60°C for ten minutes. Longitudinal section. Azan staining. Cross-striations are distinct, bending a little. Longitudinal striations are invisible. ×1500.

As is the case with cattle muscle, cross-striations of muscle fiber were notably visualized by boiling at a definite temperature. The present report deals with detailed observation on ultrastructural changes in transverse striation caused by boiling. In the unboiled muscle fiber, the isotropic disk (I disk) of a cross-striation was taller than the anisotropic disk (A disk), indicating a stage of contraction, and cross-striations were poorly differentiated (Fig. 1). Stained with azan, both disks were uniformly presented in a red tone. In 40°–muscle, A disks increased in height and became

purple with a distinct blue, while I disks were red as before. The appearance of striations became somewhat distinct in general. In 50°–muscle, A disks were purplish-blue and I disks purple, making striations more distinct. Fig. 2 shows chicken muscle boiled at 60°C for ten minutes. As is clear from the figure, transverse striations are exceedingly distinct, taking a slightly bending form, with I disks increased in height, while longitudinal striations are invisible. When stained, I disks were pale purple in color and A disks dark blue. In 70°–muscle, both disks were epually purple. In 100°–muscle, these disks became to exhibit indistinct structural and staining features.

From these observations, it is noted that, of the alternating bands in transverse striations, the I disk is decreased more rapidly in ultrastructural density than the A disk when boiled at low temperature.

d. Muscle of fish.

In ultrastructural changes of muscle fiber caused by boiling, there was an essential difference between fish and the warm-blooded animals observed. When stained with azan, chicken muscle was red before boiling, purple after boiled at 40°C, purplish-blue at 50°C, purple at 60°C, and purplish-red at 70 and 100°C. From these findings, it is indicated that the ultrastructure of fish muscle became coarse after boiling at lower temperature, or 40 to 50°C, than that of muscle of the warm-blooded animal. This characteristic difference is probably derived from the well-known fact that water soluble protein which coagulates at low temperature is more abundant in fish muscle than in mammalian and chicken muscle.

e. Observation on red and white muscle.

In comparing red and white muscle, no essential difference was noticed in ultrastructural changes between them. White muscle tended to be ultrastructurally coarse by boiling at a little lower temperature than red muscle. KAWAMURA (1954) observed in frog muscle that the ultrastructural density was low in muscle rich in myofibrils and high in muscle rich in sarcoplasm. According to WEBER (1932), myogen, the globulin complex, is more abundant in white muscle than in red muscle. Therefore, the difference between both types of muscle seemed to be derived from such different chemical composition of each muscle.

It is summarized that the boiling process had an effect of making the ultrastructure of muscle fiber coarse. In more detail, the ultrastructure of muscle fiber was not influenced by boiling at a temerature below 30°C and became the coarsest by boiling at 50 to 60°C. It was no more made coarse, however, by boiling at a temperature over 70°C, although it was damaged conspicuously by boiling. Fish muscle was influenced more rapidly by boiling at lower temperature than muscle of any other animal species examined.

2. Observation on frozen muscle.

It is generally recognized that freezing of muscle brings about characteristic changes in the structure of muscle by the formation of ice crystals and that the severity of damage varies with the method or temperature of freezing. When freezing process is carried out rapidly, ice crystals of small size are formed within and outside of muscle fibers. When freezing is done slowly, ice crystals of large size are

formed only outside of muscle fibers. In the present paper are indicated ultrastructural changes in frozen muscle.

a. Muscle under slow freezing.

Morphological changes in fish muscle frozen slowly in the refrigerator are shown in Fig. 4. As is clear from the figure, extracellular crevices are large, presenting the formation of large ice crystals along the long axis of muscle fibers. Stained with azan, muscle fibers are presented in a yellowish red tone, being more yellowish than those of unfrozen muscle. Particularly, muscle fibers adjacent to the crevices are more yellowish than those far from them. It is assumed, in this case, that the ultrastructure of muscle fiber has become dense, since it exhibited an increased affinity for small-moleculed orange G. Therefore, the muscle fibers surrounding ice crystals have a denser ultratructure than those far from them.

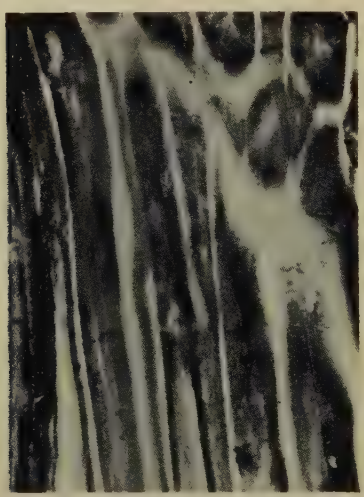

Fig. 3. Dorsal muscle of sciainoid fish unfrozen. Longitudinal section. Azan staining. ×400.

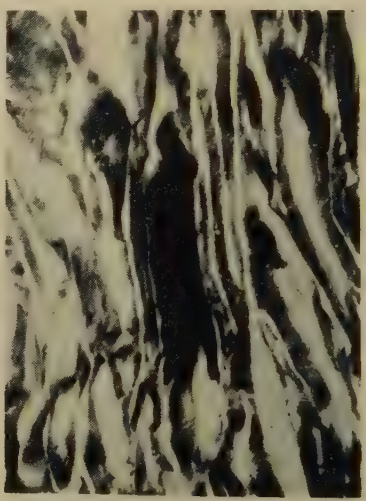

Fig. 4. Dorsal muscle of sciainoid fish frozen slowly. Longitudinal section. Azan staining. Wide intercellular spaces have been formed along the long axes of muscle fibers by the formation of large ice crystals. Disturbed fibers are in irregular arrangement. ×400.

In cattle and chicken muscle treated in the same manner, crevices among muscle fibers were smaller in size and the muscle fibers suffered less damage than in treated fish muscle. Tinctorial response to azan stain was of similar tendency to, but not so marked as, that of fish muscle.

No significant difference was noticed in ultrastructural changes between red and white muscle.

b. Muscle under rapid freezing.

Morphological changes in fish muscle frozen rapidly with a freezing microtome are illustrated in Fig. 5. Muscle tissue is severely broken down into netlike structure

by the formation of small ice crystals within and outside of muscle fibers. Stained with azan, muscle fibers are presented in a yellow tint, being obviously more yellowish than those of unfrozen control muscle and slowly frozen muscle. From these

Fig. 5. Dorsal muscle of sciainoid fish frozen rapidly. Longitudinal section. Azan staining. Muscle fibers have been broken down into a netlike structure by the formation of small ice crystals. × 400.

results, it is concluded that rapid freezing process exercised more pronounced influence on the structure and ultrastructure of muscle fiber than slow freezing process.

The tendency observed in mammalian and chicken muscle was essentially the same, but not so noticeable, as that in fish muscle.

There was no significant difference in ultrastructural changes between red and white muscle.

In short, freezing process had an effect of making the ultrastructure of muscle fiber dense, in addition to causing remarkable structural changes. This effect was more distinct in rapid freezing than slow freezing. It was more intense in fish muscle than muscle of any warm-blooded animal examined.

The ultrastructural density of muscle fiber became high after the completion of freezing process. This phenomenon would be interpreted by the removal of water from the extracellular space and muscle fibers themselves as a result of formation of ice crystals. Deprivation of water from cell elements caused reduction in size of crevices among molecules or micelles and, consequently, the ultrastructure of muscle fiber became dense.

As reported by HANDA (1950) and KAWAMURA (1952), the ultrastructural density of muscle tissue varies with animal species, age, and region of the body. So that deep consideration had been given in evaluating the results of experiments.

## (III) SUMMARY

Ultrastructural changes of skeletal muscle evoked by heat were examined micro-

scopically by means of the azan method. The specimens used had been collected from several different muscles of animals of some species. They were boiled or frozen by various methods at different temperatures.

The results obtained are summarized as follows.

1. As a whole, boiling process had an effect of making the ultrastructure of muscle fiber coarse, while freezing process had an effect of making it dense. The gradient of ultrastructural changes, however, varied to some extent with the kind and type of muscle. It is assumed that the ultrastructure of muscle became coarse by boiling, mainly because the cell elements had been coagulated and partly because they had been disintegrated, and that it became dense by freezing, because the cell elements had been dehydrated.

2. The following relationships were found between boiling temperature and ultrastructural changes of muscle fiber.

a. The ultrastructural density of muscle fiber was not changed by boiling at a temperature below 30°C and became slightly lower at 40°C and the lowest at a temperature from 50 to 60°C. The ultrastructure of muscle fiber boiled at a temperature over 70°C, however, was not any longer so coarse as that of muscle fiber boiled at 60°C. The ultrastructure of muscle fiber boiled at 100°C was coarser than that of unboiled muscle fiber.

b. White muscle fiber inclined to become coarse in ultrastructure by boiling at lower temperature than red muscle fiber.

c. Fish muscle fiber inclined to become coarse in ultrastructure more rapidly than muscle of any warm-blooded animal examined.

3. The following relationships were found between method of freezing and ultrastructural changes of muscle fiber.

a. Rapid freezing made the ultrastructure of muscle fiber denser than slow freezing.

b. Fish muscle inclined to become ultrastructurally denser through freezing process than muscle of any warm-blooded animal examined.

c. No demonstrable difference was found in the ultrastructure after freezing process between red and white muscle.

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# 畜産食品に含まれる腸球菌群に関する衛生学的研究

## 特に汚染指標としての意義について

橋　本　秀　夫
広島大学水畜産学部畜産学科

# Hygienic Studies on Enterococcus Group in Milk, Meat and Their Products, Especially on Significance as an Indicator of Pollution

Hideo HASHIMOTO[1)]
*Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan*

(Text-fig. 1 ; Tables 1–42)

(I) 緒　　言 …… 503
(II) 腸球菌群の推定培地に関する若干の検討 …… 506
(III) 各種畜産食品における腸球菌群の分布 …… 512
(IV) 人及び各種動物の糞便における腸球菌群の分布 …… 525
(V) 畜産食品及び糞便由来腸球菌群の同定分類並びに型別と由来材料との関係 …… 530
(VI) 腸球菌群の熱抵抗性試験 …… 544
(VII) 保存による腸球菌群の生残試験 …… 548
(VIII) 考　　察 …… 553
(IX) 総　　括 …… 560
(X) Summary …… 562
文　　献 …… 564

## (I) 緒　　言

食品衛生の分野において細菌学的検査を行う主要な目的は，飲食品が消化器系伝染病原菌による汚染を受けているかどうかを知ることである．しかし数多い病原菌を直接食品から検出する事は繁雑且つ時間も要するので，病原菌と常に共存し，しかも検出のより容易な菌が存在するならば，その菌を目標として調べる方が簡単であり，又病原菌による汚染の有無を類推することも可能である．この様な目的の下に調べられるのが汚染指標菌であり，食品あるいは水の検査においては従来から大腸菌群が取上げられ，既に日常検査法として検索が行われて来た．しかしながら大腸菌群の中には人畜の腸管以外にも常在する *Aerobacter aerogenes* (*Klebsiella*) などが含まれていることから，たとえ大腸菌群が検出されても必ずしも真の糞便による汚染を意味しないとの疑問が持たれるに至った．そこで大腸菌群に代るべき汚染指標菌として注目されるに至ったのが Fecal streptococci と呼ばれる腸球菌の一

1) Former address : 北海道大学獣医学部獣医公衆衛生学教室 Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

群である．本菌群も大腸菌群と同じく人畜の腸管内に存在する主要な常在菌である点において汚染指標菌たる資格を有する．

本菌群が汚染指標菌として大腸菌群よりすぐれていると注目される第1の点は，WINTER & SANDHOLZER (1946)，MALLMANN & LITSKY (1951)[48]によれば大腸菌群が人の生活や汚染と関係が無い水や土壌などから屢々検出されるのに対し，腸球菌群は糞便で汚染された水，あるいは土壌のみから検出され，人の生活と関係の無い場所からは検出されなかったといわれることであり，更に MUNDT ら (1958)[53]，堀江 (1959)[23] によってもこの事実が確かめられている．第2は汚染と平行するという点であり，MALLMANN (1928)，MALLMANN & SYPIEN (1934)[46] によればプールあるいは湖の水泳場の水を検査した時，大腸菌群は汚染の増減と関係なく出現したが，Streptococci は水泳者の増減と平行して出現したと述べられている．第3に腸球菌群は自然界において病原菌と同様死滅が早いといわれる点である．MALLMANN & LITSKY[48] は汚水で処理した土壌に *S. typhosa* を接種して生残を調べた結果，*S. typhosa* は最も早く消失し，次いで腸球菌が死滅したが，大腸菌群は長期間生残していたと述べ，又 MALLMANN & SYPIEN[46] は水泳場の Streptococci は一夜で消失したが，大腸菌群は増えることもあったと報告している．第4に人と動物の糞便由来腸球菌群の型が異るといわれる点であり，後述する様に腸球菌群の分類学的研究が進んだ結果得られた知見である．COOPER & RAMADAN (1955)[13]，BARNES & INGRAM (1955)[3]，BUTTIAUX (1958)[9]，WILSSENS & BUTTIAUX (1958)[88] は人の主要な腸球菌は *Str. faecalis* 及びその varieties であるが，ウシ，ヒツジ，ブタ等では *Str. faecium* が主要菌であると報告しており，この点は大腸菌群にみられない特性である．

さて以上の如く汚染指標菌として注目されるに至った腸球菌群も，MALLMANN (1928) がプールの汚染指標菌として Streptococci が有用であると指摘した当時は余り省みるものが無かった．それはレンサ球菌検索のための培地として，大腸菌群における様な適当にして且つ簡易な選択培地が検討されなかったためである．しかるに HARTMAN (1937) が，グラム陰性菌の発育を抑制するが，レンサ球菌の発育を阻害せぬ制菌剤として Sodium azide ($NaN_3$) が有効であることを報告して以来，Sodium azide は大いに注目されるところとなり，MALLMANN (1940) は初めてこれを液体培地に応用して糞便中の腸球菌群の菌数測定を行った．それ以来腸球菌群の検索のため Sodium azide を使用する種々の改良された選択培地が報告されるに至った．すなわち HAJNA & PERRY (1943)[16] による S.F. 培地をはじめ，PACKER (1943)[69]，WINTER & SANDHOLZER (1946)[89]，ROTHE (1948)，RITTER & TREECE (1948)[70]，LITSKY ら (1953)[44]，那須(1954)[55]，SLANETZ ら (1955[81]，1957)[82]，宮林(1957)[50] 及び堀江(1960)[27] らによる報告が行われ，腸球菌群の検索は大腸菌群同様に簡易且つ確実と認められるようになった．

以上の培地を使用して主にプール，河水，飲料水等を対象として検索[10)15)27)37)42)43)44)45)70)81)82)84)]が行われた結果，水の衛生学的検査においては糞便汚染指標菌としてそれ迄行われて来た大腸菌群と共に，あるいはそれに代るべき汚染指標菌としてますます注目され，既に英国においては日常検査に本菌群の検索が取入れられているといわれている[43)45)]．

上述の如く水を対象とした検索が行われる一方，食品衛生の面においても本菌群が大腸菌群に代る汚染指標菌として意義を有するか否かが検討された．しかも初めは水の汚染と関係ある水産食品を対象とした報告が多く，OSTROLENK ら (1947)[68]はカニその他，MCCLESKEY

& Boyd (1949)[49]はカニ，Wilson & McCleskey (1951)[86][87]，坂井 (1956)[71]はカキ，那須(1954)[56]がイカ・タコ，Larkin ら (1956)[41]が冷凍魚類，柳沢ら(1954)[90]，宮林(1957)[51]，堀江(1960)[27]が魚介類その他について腸球菌群を検索し，更に大腸菌群との出現を比較しながら検討を行っている．

他方畜産食品において乳及び乳製品については腸球菌が存在する事実は早くから知られていた．Sherman & Niven (1938)[78]，Beahm (1942)[7] は生乳あるいは殺菌乳，Nicholus (1939)[57]は粉乳における分離レンサ球菌を同定して腸球菌の存在を報告しているが，1943年に至り Hajna & Perry[16]は初めてS.F. 培地を用いて生乳の腸球菌を，White & Sherman (1944)[85] はペニシリンと Sodium azide を含む選択培地を用いて生乳及び殺菌乳の腸球菌を検査し，Kosikowsky & Dahlberg (1948)[40] はチーズ中の *Str. faecalis* 分離のため同様の培地を使用するなどの報告が次第にみられるに至った．我国においても井上(1955)[32]がバター，八田ら(1956)[22]が生乳及び市乳，大嶋(1956)[65]がチーズ，青木ら(1957)[1] が粉乳及びミックスパウダーについて，著者ら(1958[17]，1959)[21]も各種乳製品における検査成績並びに分離腸球菌の性状についての報告を行った．一方近年に至り肉及び肉製品の腸球菌についても検索が行われ，Niven (1952) はレンサ球菌が肉に存在する主要な菌であると述べている．又 Barnes & Ingram (1955)[3][31]，Niven (1955)[58] は缶詰ハムの腸球菌を検査して汚染の由来を論じた．更に Kereluk & Gunderson (1959)[34]，Hartman (1960)[18] は冷凍した肉パイについて腸球菌の分布，汚染を調査し，Kereluk (1959)[35] は分離腸球菌の同定報告を行っている．我国においても市川・国田(1952)[29]は食中毒菌としての見地から生肉及び肉製品における本菌の分布を調査し，宮林(1957)[51]は牛肉における腸球菌の夏期と冬期との分布の差を検討した．

水あるいは各種食品における腸球菌群の分布状態は次第に明らかにされて来たが，それら報告の多くは汚染の度合，すなわち陽性率並びに出現菌数を調べたものが多く，更に進んで材料と出現菌型との関係迄検討したものは非常に少ない．

Bergey's Manual (1957)[8]には Enterococcus group として，*Str. faecalis, Str. faecalis* var. *liquefaciens, Str. faecalis* var. *zymogenes* 及び *Str. durans* の4型が記載されているが，一部にはこの分類を不十分として *Str. faecalis* を更に *Str. faecalis* と *Str. faecium* なる2種に区別する主張がなされている．*Str. faecium* なる種名は Orla-Jensen (1919) が Lactic acid bacteria の分類学的研究で記述した事に始まっている．その後腸球菌群の分類に関しては，Sherman (1937[77]，1938)[79] の詳細な研究があり，上記4菌種が記載されているが，*Str. faecium* を区別していない．しかるに1950年に至り，Skadhauge[80] は人由来の腸球菌群の分類を試みた際，Potassium tellurite ($K_2TeO_3$) に対する感受性の差から *Str. faecalis* と *Str. faecium* とは異った菌種として取扱うべきであると報告した事に始まり，Sharpe & Shattock (1952)[75]，Shattock (1955)[76] も *Str. faecium* を *Str. faecalis* から分けられるという説を支持し，又 Seelemann (1954)[74] は *Str. faecium* をD群レンサ球菌の代表菌種として記載している．更に1956年 Barnes[4]は TTC (2・3・5-triphenyltetrazolium chloride) に対する腸球菌群の作用を調べて，*Str. faecalis* 及びその varieties と *Str. faecium* との間には還元性に差がある事を認め，この点からも *Str. faecalis* と *Str. faecium* とは型別出来ると主張するに至った．その後以上の分類型式を採用する報告[5][6][35]も現われ，Barnes & Ingram (1955)[3]は缶詰ハムについて，Moutoussis ら (1957)[52] は殺菌乳につ

いて分離腸球菌群を同定した結果 *Str. faecium* が多かったと述べ，又 MUNDT ら (1958)[53] は植物類その他，BUTTIAUX (1958)[9]，WILSSENS & BUTTIAUX (1958)[88] は糞便，BUTTIAUX (1958)[10]，LECLERC & CATSARAS (1958 59)[42] は飲料水の検査においても *Str. faecium* が最も多く出現したと報告している．著者は Bergey's Manual 及び以上の報告を参考として，各種の材料から分離した腸球菌群の分類を行い，更に検査材料と出現菌型との関係についても検討を行った次第である．

畜産食品における腸球菌群の分布に関しては上述の如くかなり多くの報告がみられるが，これらを詳細に検討すると報告の多くは単に腸球菌群の定性的報告のみで定量的検査迄は行われておらず，又あっても検査例数及び種類が少なく，しかも原料から製品に至る系統的な調査研究は殆んど行われていない．勿論汚染指標菌としての意義迄深く追及した報告は殆んど見当らない．よって著者は本邦産の畜産食品を材料として腸球菌群の定性的並びに定量的試験を行い分布状態を明らかにすると共に，分離腸球菌群の各種生物学的性状を調べて型別し，更にその成績を基として種々の角度から汚染指標菌としての意義を検討した．その結果畜産食品における腸球菌群の汚染指標菌としての意義は大腸菌群と同様に，又ある場合にはそれ以上に意義ある事を認めたので以下その成績について報告する．

## (II) 腸球菌群の推定培地に関する若干の検討

著者ら[17]が報告した如く，乳製品の腸球菌群検査に当っては那須[55]の処方による選択培地を使用した．本培地は Sodium azide ($NaN_3$) を 0.02% に含む推定培地 (B.T.B. Azide Dextrose Broth) 及び 確認培地 (B.T.B. Azide Dextrose Agar) からなっており，乳製品の如き加熱工程を経た一般生菌数の少ない材料にあっては腸球菌群以外の他種菌の発育が認められず，検査にあたり何等支障が無かったことは既に報告[21]したとおりである．しかし生乳，生肉等の如く製品に比べて菌の種類及び生菌数が圧倒的に多い非加熱材料の検査に当っては，腸球菌群以外のレンサ球菌，ブドウ球菌並びに芽胞形成菌等の発育する例もみられ本培地の選択能力に不十分な点があることを認めた．よってこれらの点を改良するため，$NaN_3$ の濃度を増し更に近年芽胞形成菌の抑制剤として用いられている E.V. (Ethyl violet)[25][26][44][50][91][92] を加えた推定培地について，腸球菌群及び他種菌を供試して，腸球菌群のみの発育を許して他種菌の発育を抑制出来るかどうか，又腸球菌群自体もどの程度抑制を受けるかどうかの 2 点について若干の比較検討を試みた．

### I 実験材料及び方法

#### 1. 培　　地

Table 1-1 に示す如き組成の基礎培地に Sodium azide (鹿印 1 級) の濃度を 0.02, 0.025, 0.03% に加えたもの及び E.V. (Bayer) を加えた培地を用意した．すなわち Table 1-2 に示すように培地 No. 1 は基礎培地に Sodium azide を 0.02% になるよう加えたものであり，B.T.B. Azide Dextrose Broth として既に乳製品の検査に使用して来たものである．培地 No. 2 及び No. 3 は基礎培地に Sodium azide をそれぞれ 0.025 及び 0.03% になるように加えたもの，培地 No. 4 は培地 No. 3 に更に E.V. を 0.00006% に加えたものである．E.V. の濃度は宮林[50]の報告に準じた．なお対照として基礎培地のみのものを用意した．これらの培地は 2 ml づつ小試験管に分注して滅菌された．確認培地として用いた B.T.B. Azide Dextrose Agar は那須の処方における肉エキスを酵母エキス (大五栄養化学) で置換え，又 Sodium

azide の濃度を培地 No. 2 と同じくした．すなわち Table 1-3 に示すように培地 No. 2 に酵母エキス (0.5%) 及び粉末寒天 (1.5%) を加えた培地である．

Table 1. Composition of screening media for detection of enterococcus group.

1 Basal medium

| | | |
|---|---|---|
| Polypepton | 20g | |
| Dextrose | 10g | |
| NaCl | 5g | pH7.0 |
| Brom thymol blue | 0.032g | |
| Distilled water | 1,000ml | |

2 Presumptive medium

| Division | Ingredient of media |
|---|---|
| Control | Basal medium |
| No. 1 | 〃 〃 + Sodium azide (0.02%) |
| No. 2 | 〃 〃 + 〃 〃 (0.025%) |
| No. 3 | 〃 〃 + 〃 〃 (0.03%) |
| No. 4 | No. 3 + Ethyl violet (0.00006%) |

3 Confirmatory medium

Presumptive medium (No. 2) + Yeast extract (0.5%) + Agar (1.5%)

## 2. 供 試 菌 株

供試菌株の一覧は Table 2 に示すとおりであって，各種腸内細菌群を主体として *Bacillus*, *Micrococcus*, *Streptococcus*, Enterococcus, その他腐敗に関与する菌，酵母等 96株を供試した．Enterococcus, *Micrococcus*, 腸内細菌群及び *Bacillus* の 1 部とその他若干は教室保存株を使用したが，*Str. lactis*, 酵母及び *Bacillus* の 1 部は北大農学部応用菌学教室佐々木教授より，腸内細菌群の 1 部は農林省家畜衛生試験場波岡博士より分与されたものである．

Table 2. The list of organisms used.

| Organism | No. of strains | Organism | No. of strains |
|---|---|---|---|
| *Salmonella* | 5 | *Serratia* | 2 |
| *Arizona* | 1 | *Pseudomonas* | 1 |
| *Escherichia* | 4 | *Achromobacter* | 1 |
| *Citrobacter* | 5 | *Bacillus* | 6 |
| *Klebsiella* | 3 | *Saccharomyces* | 1 |
| *Cloaca* | 3 | *Hansenula* | 1 |
| *Hafnia* | 3 | *Micrococcus* | 13 |
| *Proteus* | 3 | *Staphylococcus* | 7 |
| *Morganella* | 3 | *Str. lactis* | 5 |
| *Rettgerella* | 3 | *Str. bovis* | 5 |
| *Providencia* | 3 | Enterococcus | 15 |

## 3. 実験方法及び判定基準

供試菌のブイヨン 37°C 20時間培養菌液を 1 滴づつ各推定培地並びに対照培地に接種し，

37°C 48時間培養を行って発育態度を観察した．すなわち黄変，混濁，沈澱を示したものは＋，混濁，沈澱のみで黄変微弱なものは±，混濁も沈澱もみられないもの，あるいは混濁か沈澱のみを示すが黄変しないものは－と判定した．推定培地に発育した時は更に確認培地に画線培養を行って37°C 48時間培養後その発育の有無を確認した．なお確認培地上で黄変集落が発育した時は＋，微小な黄変集落は±，集落を形成するが黄変しないもの及び痕跡程度の発育は－と判定した．

一方 Table 2 に示した *Str. bovis* 5株及び Enterococcus 15株（生肉，粉乳及び糞便由来各5株）を特に取上げ，既報[21]の培養温度を異にした際の発育の比較実験と同様の方法で各推定培地における発育態度を比較検討した．まず供試菌のブイヨン20時間培養菌液を1,000

Table 3-1. Growth of *Enterobacteriaceae*, *Bacillus* and others in various presumptive media.

| Organism | No. of strains | Growth in various presumptive media | | | | | Growth on confirmatory medium |
|---|---|---|---|---|---|---|---|
| | | Control | No. 1 | No. 2 | No. 3 | No. 4 | |
| *S. enteritidis* | 1 | ＋ | ±～－ | ±～－ | － | － | － |
| *S. typhimurium* | 1 | ＋ | － | － | － | － | |
| *S. choleraesuis* | 1 | ＋ | － | － | － | － | |
| *S. thompson* | 1 | ＋ | － | － | － | － | |
| *S. pullorum* | 1 | ＋ | － | － | － | － | |
| *Arizona* | 1 | ＋ | ＋ | ± | － | － | － |
| *Escherichia* | 4 | ＋ | － | － | － | － | |
| *Citrobacter* | 2 | ＋ | ± | ± | ± | ± | － |
| 〃 | 1 | ＋ | ±～－ | － | － | － | |
| 〃 | 2 | ＋ | － | － | － | － | |
| *Klebsiella* | 3 | ＋ | － | － | － | － | |
| *Cloaca* | 3 | ＋ | － | － | － | － | |
| *Hafnia* | 3 | ＋ | － | － | － | － | |
| *Prot. vulgaris* | 3 | ＋ | － | － | － | － | |
| 〃 *mirabilis* | 3 | ＋ | － | － | － | － | |
| *Morganella* | 3 | ＋ | － | － | － | － | |
| *Rettgerella* | 3 | ＋ | － | － | － | － | |
| *Providencia* | 3 | ＋ | － | － | － | － | |
| *Serratia* | 1 | ＋ | ＋ | ± | ± | ± | － |
| 〃 | 1 | ＋ | ±～－ | ±～－ | － | － | |
| *Pseud. fluorescens* | 1 | ＋ | － | － | － | － | |
| *Achromobacter* | 1 | ＋ | － | － | － | － | |
| *B. mesentericus* | 2 | ＋ | － | － | － | － | |
| *B. subtilis* | 2 | ＋ | － | － | － | － | |
| *B. megaterium* | 1 | ＋ | － | － | － | － | |
| *B. cereus* | 1 | ＋ | － | － | － | － | |
| *Sacch. cerevisiae* | 1 | ± | － | － | － | － | |
| *Hansenula anomala* | 1 | ± | － | － | － | － | |

Notes. ＋ : Growth.
± : Weak growth.
－ : No growth.

倍で辛うじて検出される程度に生理食塩水で稀釈したものを原液とし，更にこれを4段階に稀釈して各推定培地及び対照培地毎に各段階それぞれ5本づつ接種培養を行った．接種量は各管 0.2ml 宛とし，これらを 37°C に培養して 24，48 及び 72時間後における発育態度を比較した．

## II　実　験　成　績

### 1.　腸球菌群と他種菌の発育態度

*Enterobacteriaceae*, *Bacillus*, その他腐敗関与菌，酵母等の各推定培地及び対照培地における発育態度は Table 3-1 に示すとおりである．対照培地には供試株すべてが発育した．但し酵母では沈澱が多量に出現したにもかかわらず，混濁，黄変は微弱であった．推定培地には，*S. enteritidis*, *Arizona*, *Citrobacter* 及び *Serratia* の1部が幾分発育を示した他はすべて陰性であった．又推定培地で発育したものは確認培地で非発育あるいは発育してもその集落は痕跡程度で黄変する迄に至らないため腸球菌と見あやまる恐れは無かった．なお *Bacillus* は培地 No. 1 においてすら全く発育は認められなかった．

*Micrococcus* 及び *Streptococcus* の発育態度は Table 3-2 に示す如く，*Sta. aureus* 及び *Sta. albus* の1部及び *Str. lactis* が陰性の他は程度に差はあるがすべて推定培地に発育した．*Micrococcus* はコアグラーゼテストの陰陽にかかわらず培地 No. 2 に発育し，*Sta. aureus* の2株，*Sta. citreus* の1株は培地 No. 3 に発育した．*Str. bovis* は培地 No. 2 では

Table 3-2.　Growth of *Micrococcus* and *Streptococcus* in various presumptive media.

| Organism | No. of strains | Growth in various presumptive media | | | | | Growth on confirmatory medium |
|---|---|---|---|---|---|---|---|
| | | Control | No. 1 | No. 2 | No. 3 | No. 4 | |
| *Micrococcus* (Coag. +) | 8 | + | + | + | − | − | − |
| 〃 ( 〃 −) | 4 | + | + | + | − | − | − |
| 〃 ( 〃 −) | 1 | + | + | ± | − | − | − |
| *Sta. aureus* | 1 | + | + | + | ± | − | − |
| 〃 〃 | 1 | + | + | ± | ± | − | − |
| 〃 〃 | 1 | + | − | − | − | − | |
| 〃 *albus* | 1 | + | ± | − | − | − | |
| 〃 〃 | 2 | + | − | − | − | − | |
| 〃 *citreus* | 1 | + | + | + | ± | − | − |
| *Str. lactis* | 5 | + | − | − | − | − | |
| 〃 *bovis* | 2 | + | + | + | − | − | − |
| 〃 〃 | 1 | + | + | + | ± | − | − |
| 〃 〃 | 1 | + | + | + | + | − | − |
| 〃 〃 | 1 | + | + | + | + | + | ±〜− |
| 〃 *faecalis* | 2 | + | + | + | + | + | + |
| 〃 *durans* | 1 | + | + | + | + | + | + |
| 〃 *faecium* | 6 | + | + | + | + | + | + |
| 〃 *faecalis* var. *liquefaciens* | 6 | + | + | + | + | + | + |

Notes.　+ : Growth.
± : Weak growth.
− : No growth.

全株，No. 3 に 3 株，No. 4 に 1 株が発育した．しかしこれら推定培地に発育した株も確認培地では *Str. bovis* の 1 株が ±～－ を示した他はすべて発育出来なかったのに対し，腸球菌群の15株は全株が推定及び確認培地に完全に発育した．以上の如く *Str. bovis* の中には培地 No. 3 及び No. 4 で＋，確認培地においても ± あるいは痕跡程度に発育し，腸球菌群に最も近い発育態度を示したので，更に両者を取上げて実際の検査時における如く微量な菌量が接種された際の発育態度を比較するため次の如き実験を行った．

2. 腸球菌群及び *Str. bovis* の培地及び接種菌量の差による発育の比較

腸球菌群（15株）及び *Str. bovis*（5 株）の微量菌を各培地に接種して発育を比較した成績は Table 4 に示すとおりである．培地の試験管数は各培地稀釈段階毎に 1 株当り 5 本づつ使用した．

腸球菌群において24時間培養では対照培地に比べて各推定培地における発育管数は少なく，特に培地 No. 4 では接種菌液の原ですら抑制されるのが目立ち，計においては対照培地のわずか 30.4%（$^{64}/_{210}$）の発育を示すに過ぎない．培養48時間目において，各推定培地での発育管数の増加は対照培地のそれより多くなり，特に培地 No. 4 では著明であったが，それら発育管数の計は培地 No. 1～4 の順に対照培地の 98.1%（$^{211}/_{215}$），93.4%（$^{201}/_{215}$），90.2%（$^{194}/_{215}$）及び 80%（$^{172}/_{215}$）という発育成績であった．更に培養 72時間目における発育管数の増加は対照及び No. 3, 4 に軽度にみられたが，No. 1, 2 では変化が無かった．

一方 *Str. bovis* にあっては腸球菌群に比べて明らかに抑制の影響を受けている事が認められる．すなわち24時間培養では培地 No. 1 においてさえ対照培地の半分以下，培地 No. 4 では原においてすら陰性であった．培養48時間目において各培地における発育管数は非常に増加したが，それでも Sodium azide 及び E.V. による発育抑制の影響が明らかであり，特に培地 No. 4 では依然として全例陰性に止まっている．培養72時間目においては対照培地での発育管数の増加はみられなかったが，その他の培地ではすべて増加し，培地 No. 4 においては初めて発育陽性管が認められるに至った．以上の成績から *Str. bovis* は腸球菌群に比べて，Sodium azide に対する感受性がより鋭敏であることが認められた．すなわち 前項において *Str. bovis* は培地 No. 3 及び 4 では＋～－の発育を示していたが，この成績は 今回の No. 3 及び 4 の培地における *Str. bovis* の発育が，菌数が多い時又は培養時間が長ければ発育を来し，菌数が少ない時又は培養時間が短かければ発育不良を示したことによっても明らかになし得たものと考えられる．

## Ⅲ 小　　括

実験成績 1 に示した如く腸球菌群及び他種菌を供試株として Sodium azide 含有量を異にし，あるいは Ethyl violet を加えた各推定培地における発育を検討した結果，腸球菌群はすべて＋に，*Str. bovis* は 5 株中 1 株が同程度に発育した．又 *Micrococcus, Staphylococcus, Arizona, Citrobatcer, Serratia* 等は＋～±程度で発育したが，その他多くの菌種は発育するに至らなかった．なおこれら推定培地で発育陽性を示したものも，確認培地の段階では腸球菌群がすべて＋，*Str. bovis* の 1 株が ±～－を示した 以外は発育陰性を示した事から，腸球菌群とは 簡単に区別される事が 判明した．すなわち 培地における Sodium azide の濃度が 0.02%（培地 No. 1）では腸球菌群以外の菌でも 発育可能であるが，0.025%（培地 No. 2）では腸球菌群以外の菌で発育するものがあっても確認培地の段階では抑制することが出来る．但し 例外として *Str. bovis* で ±～－に 発育するものもある．又培地 No. 3 (Sodium azide

Table 4. Comparison of growth of enterococcus group and *Str. bovis* to different inoculating doses in various presumptive media.

| Organism | Medium | Incubation hours (37°C) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 | | | | | 48 | | | | | 72 | | | | |
| | | Density of inoculated culture | | | | Total (b/a)* | Density of inoculated culture | | | | Total (b/a)* | Density of inoculated culture | | | | Total (b/a)* |
| | | Original | ×10 | ×100 | ×1,000 | | Original | ×10 | ×100 | ×1,000 | | Original | ×10 | ×100 | ×1.000 | |
| Enterococcus group | Control | 75 | 67 | 51 | 17 | 210/300 | 75 | 69 | 52 | 19 | 215/300 | 75 | 72 | 53 | 19 | 219/300 |
| | No. 1 | 75 | 60 | 43 | 9 | 187 | 75 | 70 | 51 | 15 | 211 | 75 | 70 | 51 | 15 | 211 |
| | No. 2 | 75 | 63 | 38 | 9 | 185 | 75 | 69 | 45 | 12 | 201 | 75 | 69 | 45 | 12 | 201 |
| | No. 3 | 75 | 55 | 35 | 6 | 171 | 75 | 67 | 43 | 9 | 194 | 75 | 69 | 44 | 9 | 197 |
| | No. 4 | 39 | 19 | 6 | 0 | 64 | 65 | 59 | 40 | 8 | 172 | 71 | 64 | 43 | 8 | 186 |
| *Str. bovis* | Control | 25 | 25 | 17 | 7 | 74/100 | 25 | 25 | 18 | 9 | 77/100 | 25 | 25 | 18 | 9 | 77/100 |
| | No. 1 | 15 | 11 | 5 | 2 | 33 | 25 | 19 | 14 | 4 | 62 | 25 | 23 | 18 | 5 | 71 |
| | No. 2 | 10 | 5 | 3 | 0 | 18 | 20 | 16 | 11 | 4 | 51 | 25 | 20 | 13 | 4 | 62 |
| | No. 3 | 10 | 1 | 0 | 0 | 11 | 15 | 10 | 7 | 3 | 35 | 20 | 14 | 9 | 3 | 46 |
| | No. 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 4 | 4 | 2 | 16 |

* Denominator (a) : No. of tubes inoculated.
Numerator (b) : No. of tubes grown.

0.03%) 及び No. 4 (No. 3 +E.V.) において腸球菌群は全株，*Str. bovis* は 1 部が発育したがその他の菌はすべて抑制された．

以上から *Str. bovis* が最も腸球菌群に近い発育態度を示す事を知ったので，又腸球菌群自体もどの程度抑制を受けるかどうかをみるため両者を取上げて実際の検査時における如く微量な菌を接種して発育性を検討した結果，実験成績 2 に示すとおり，両種菌の Sodium azide による影響がかなり著明に現われ，培養 72時間目において腸球菌群の発育は培地 No. 1 では対照の 4 %減，No. 2 では 8 %減，No. 3 では10%減と培地の Sodium azide 量が増加するにつれて発育不良となり，更に E.V. を添加した No. 4 では15%減の成績であった．他方 *Str. bovis* は腸球菌群よりも更に強く影響を受け，培地 No. 1 では 8 %減，No. 2 で19%，No. 3で 40%，No. 4 では実に79%も抑制された．又この様な影響は菌数の少ない程著明であったが，反面培養時間の延長に伴なって発育して来る傾向も認められた．

結局可検材料より腸球菌群を定量的に検出する際の推定培地としては，Sodium azide の含有量は 0.02%でも大体目的を達し得るが，*Micrococcus, Staphylococcus, Str. bovis* の如き菌種に対しては 0.03%が好ましい．しかし確認培地での Sodium azide 量は0.025%であるが腸球菌群以外の 菌種の発育を 抑えている点などを 考慮すると，一般の菌種に 対しては 0.025%とするのが妥当の様である．しかし 著者ら[21]が報告したように 乳製品の如き加熱食品で一般生菌数の少ない材料の検査に当っては，0.02%で十分と考えている．又培養時間は 48時間が適当である．なお Ethyl violet の培地への添加はその必要性を認めない．

### (III) 各種畜産食品における腸球菌群の分布

前章の培地並びに培養方法によって各種畜産食品における腸球菌群の定性的並びに定量的検査を行って腸球菌群の分布状態を調査した．なお汚染指標菌としての腸球菌群に関する報告には，大腸菌群の検査も同時に行って両者の菌群の汚染状態を比較検討しているものが多いので，著者もこれらにならって大腸菌群の検索も行ったが，更に一般生菌数の検査も行って 3 者の出現状況から汚染の検討を試みた．

#### I 検 査 材 料

1. 乳房直接採取乳

本材料は10戸の酪農家で飼育されていた50頭の牛について各分房から，初めの数搾りは棄てた後無菌的に直接試験管に採取した乳 200例である．材料の蒐集期間は 1959年 5 月 7 日から 6 月 7 日にわたっており，材料はすべて採取後 2 時間以内に実験室に運ばれて検査に供された．

2. プラント持込乳

本材料は1959年 6 月16日から同月25日迄の間に札幌市内の 1 ミルクプラントに持込まれた近郊 7 地区由来の輸送缶50缶より採取されたものである．これらは直ちに氷塊の入った魔法びんに納められて冷却され，1 時間以内に実験室に運ばれて検査に供された．

3. 乳製品 (1957年度検査分)

本材料は脱脂粉乳14，調製粉乳13，全粉乳及び加糖粉乳各 2，ミックスパウダー 6，加糖練乳17，プロセスチーズ 7 及びバター44 の計 105材料であり，何れも1956年及び1957年製である．メーカーはバターのみが14メーカーにわたっているが，その他の製品は 3 大メーカー製である．検査は何れも製造後 6 カ月以内の期間に行われた．

4. 粉乳（1958年度検査分）

本例は北海道内5カ所の乳製品工場から送られて来た材料で，1958年10月1日から同月31日の間に製造されたものをA工場では7～19日，B工場では16～27日の如く連続採取した脱脂粉乳69，全粉乳12計81材料である．検査は製品の製造後すべて1月以内に行われた．

5. 生　　肉

本材料は札幌市内の百貨店及び精肉小売店から購入した豚肉及び牛肉で，1959年の冬期（1月26日～2月24日）は豚肉，牛肉各30例，夏期（8月15日～8月22日）は豚肉，牛肉各20例の計100材料である．これらの材料は店頭で既にうすく切られており，経木に包まれたままの状態で購入後（夏季は魔法びんを用いて）1時間以内に実験室に運ばれて試験に供された．なお購入店は冬季32軒，夏季22軒であり，このうち夏冬2回共購入した店は11軒であった．

6. 肉　製　品

本材料は札幌市内の4つの百貨店（1材料のみ精肉小売店）から1960年8月27日より10月4日の間に購入した8メーカー製のドメスチックソーセージ7，ドライソーセージ13，ハム17及びベーコン2の計39材料である．ソーセージ類はメーカー包装のまま，ハムは適当な大きさに分割された塊りで購入された．又ベーコン及びスモークハムの各2例はスライスされた真空包装品である．これらの材料は購入後0°Cの冷蔵庫に保存された後試験に供された．製造月日の標示のあったもの30例，無いもの9例（ドライソーセージ7，ベーコン2）である．製造月日の標示品では製造後2カ月の1例を除くほかは3週間以内で検査に供された．

## II 検 査 方 法

各材料について生乳はそのままを原液とし，乳製品は食品衛生検査指針[38]に従い共栓の稀釈びんを用いて5あるいは10倍稀釈液を調製し，これを原液とした．生肉は5gを秤量してホモジナイザーのカップに取り，生理食塩水45mlを加えてホモジナイザーにかけ，均質化した10倍稀釈液を原液とした．肉製品は包装を開き深部より10gを秤取して生肉同様ホモジナイザーにかけた5倍液を原液とした．以上の各原液について更に段階稀釈液を作り，菌数の測定に供した．すなわち各材料共腸球菌群数と共に検査指針に準じて一般生菌数及び大腸菌群数をも測定した．但し乳房直接採取乳のみは後2者を省略して腸球菌群数のみを測定した．

腸球菌群数の検査に当っては（II）章で述べた培地No.1の処方による推定及び確認培地を用いた．但しプラント持込乳，夏季検査の生肉及び肉製品の1部については培地No.2を使用した．すなわちこれら推定培地を10mlづつ分注した各段階それぞれ5本の中試験管に各稀釈液を1mlづつ接種して37°Cに48時間培養後，発育の疑わしい管については72時間迄培養し，黄変混濁したものを推定試験陽性とした．更にこの陽性管から確認培地に画線培養して37°C 48時間培養後，黄変集落の発育したものを確定試験陽性とした．ついでこれら陽性を示した材料について改めて推定培地から血液寒天平板に培養を行い，発育した集落を観察して異ると思われる代表集落から菌株を分離した．更にこれら分離株については（V）章に後述する如き同定試験を行い腸球菌と同定後，改めて推定培地における成績からM.P.N.法により腸球菌群数を算定した．なお1957年度検査の乳製品のみは既報[21]に示した如く0.2mlづつ5本の推定培地に接種する方法で算定を行った．

大腸菌群数についてプラント持込乳はDesoxycholate Agar（栄研）を，1957年度検査の乳製品はViolet Red Bile Agar (Difco) 使用したが，その他は各段階稀釈液1mlづつを5本のB.G.L.B. 醱酵管に接種し以下検査指針に従い完全試験迄行った後M.P.N.値を求めた．

一般生菌数は乳及び乳製品については乳製品用標準寒天培地（栄研），肉及び肉製品については酵母エキス寒天培地（栄研）を用いて測定した．なおプラント持込乳については同時に総菌数 (Breed 値，個体法) をも求めて比較を行った．

Table 5. Most Probable Number (M. P. N.) of enterococcus group bacteria per ml in directly milked samples from each quarter of bovine udder.

| Cow No. | Site of udder | | | |
|---|---|---|---|---|
| | Left anterior | Right anterior | Left posterior | Right posterior |
| 41 | 1.3 | 0.8 | — | — |
| 42 | — | 0.4 | — | — |
| 43 | — | — | 0.5 | 0.2 |
| 49 | — | — | — | 0.4 |

Table 6. Numbers of enterococcus group bacteria, coliform bacteria and living bacteria per ml in the raw milk sampled at plant.

| Sample No. | Place of milking | Enterococcus group bacteria (M.P.N. per ml) | Plate count per ml | | Sample No. | Place of milking | Enterococcus group bacteria (M.P.N. per ml) | Plate count per ml | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Coliform bacteria[1] | Living bacteria[2] | | | | Coliform bacteria[1] | Living bacteria[2] |
| 1 | A | 3.3 | 10 | 12 | 26 | E | 280 | 840 | 250 |
| 2 | 〃 | 24 | 210 | 160 | 27 | 〃 | 0.8 | 32 | 16 |
| 3 | 〃 | 3.3 | 10 | >30 | 28 | 〃 | 0.4 | 5 | 42 |
| 4 | 〃 | 33 | 50 | >20 | 29 | 〃 | 0.5 | 20 | 18 |
| 5 | 〃 | 43 | 70 | >45 | 30 | 〃 | 0.2 | 120 | 38 |
| 6 | B | 1.7 | 220 | >38 | 31 | F | 3.1 | 4,300 | 2,000 |
| 7 | 〃 | 0.4 | <10 | >12 | 32 | 〃 | 79 | 15 | 38 |
| 8 | 〃 | 2.6 | 40 | >30 | 33 | 〃 | 350 | 220 | 210 |
| 9 | 〃 | 130 | <10 | >28 | 34 | 〃 | 0.7 | <10 | 140 |
| 10 | 〃 | 4.9 | <10 | >16 | 35 | 〃 | 17 | 10 | 250 |
| 11 | C | 16,000 | 500 | >350 | 36 | 〃 | 4.9 | 10 | 12 |
| 12 | 〃 | 3,500 | 3,300 | 90 | 37 | 〃 | 79 | 440 | 4,000 |
| 13 | 〃 | 2,400 | 6,800 | >230 | 38 | 〃 | 79 | 40 | 1,500 |
| 14 | 〃 | 9,200 | 11,000 | 300 | 39 | 〃 | 110 | 5 | 8 |
| 15 | 〃 | 9,200 | 1,300 | 310 | 40 | 〃 | 3.3 | 5 | 48 |
| 16 | 〃 | 79 | 610 | 62 | 41 | G | 4.9 | <10 | 250 |
| 17 | 〃 | 79 | 1,500 | 140 | 42 | 〃 | 1,600 | 142,000 | 130 |
| 18 | 〃 | 24 | 110 | 90 | 43 | 〃 | >1,800 | 30 | 750 |
| 19 | 〃 | 22 | 95 | 85 | 44 | 〃 | 3.3 | 110 | 110 |
| 20 | 〃 | 4.9 | 1,200 | 70 | 45 | 〃 | 1.7 | 40 | >800 |
| 21 | D | 790 | 840 | 400 | 46 | 〃 | 54 | 2,100 | 160 |
| 22 | 〃 | 220 | 440 | 45 | 47 | 〃 | 0.5 | 30 | 30 |
| 23 | 〃 | 0.5 | 1,800 | 240 | 48 | 〃 | 28 | 390 | 350 |
| 24 | 〃 | 0.2 | 75 | 1,300 | 49 | 〃 | 1.7 | 55 | 250 |
| 25 | 〃 | 0.4 | 120 | 46 | 50 | 〃 | 33 | 70 | 65 |

Notes. 1) Medium : Desoxycholate agar (Eiken).
2) Unit : 10 thousand.

## Ⅲ 検 査 成 績

### 1. 乳房直接採取乳

50頭の乳牛の各分房より搾乳した計200例における腸球菌群の陽性率はわずかに3%(6/200)であり極めて低率であった．なおこの陽性6例中5例は同一牛舎の3頭に出現したものである．本例の腸球菌群数は Table 5 に示すように 1 ml 当り 0.2～1.3 と非常に少ない数値を示した．

なお本材料における腸球菌群陽性例は少なかったが，腸球菌群以外のレンサ球菌あるいはブドウ球菌は，例数，菌量共腸球菌群よりはるかに多く検出された．

### 2. プラント持込乳

輸送缶から採取した50例についての成績は Table 6 の如くである．腸球菌群及び大腸菌群は100%陽性であった．腸球菌群数は 1 ml 当り 0.2～16,000，大腸菌群数は 5～142,000の範囲に分布していた．個々の材料について腸球菌群と大腸菌群の出現菌数を比較すると Table 7 の如く後者の菌数の多い方が37例で多数を占める成績であった．

Table 7. Comparison of numbers of enterococcus group bacteria and coliform bacteria in raw milk samples from plant.

| Appearances | No. of samples |
|---|---|
| Enterococcus group bacteria less than coliform bacteria | 37 |
| 〃 〃 〃 more 〃 〃 〃 | 9 |
| Not compared | 4 |

一般生菌数は ml 当り 8万～4,000万に分布し，400万以下44例 (88%) でわが国における原料乳としては良好なものが多かった．同時に行った Breed 値（個体法）との関係は Table 8 の如くであり，大体平行しているのが観察される．

Table 8. Relationship of plate count and direct microscopic count (individual count) in 50 raw milk samples from plant.

| Division | Range of bacterial count per ml | | | | |
|---|---|---|---|---|---|
| | Under 10 thousand | ～1 million | ～4 million | ～10 million | Over 10 million |
| Plate count | 1 | 25 | 18 | 2 | 4 |
| Breed method | 1 | 19 | 15 | 13 | 2 |

### 3. 乳製品（1957年度検査分）

製品8種計105材料についての検査成績は Table 9 に示されるが，本表については既に報告[17]しているので簡単な記載に止める．腸球菌群は練乳，プロセスチーズを除く他製品41例から検出され，大腸菌群陽性は5例でバターのみにみられた．一般生菌数5万以上は9例でこれもバターのみにみられた．なおこのうちの8例は腸球菌群も陽性であり，更にそのうちの5例は大腸菌群も陽性であった．腸球菌群の陽性率は 39.0%(41/105) であったが，大腸菌群のそれは 4.7%(5/105) であり，前者の陽性率が遥かに高い．各製品における個々の陽性率は大腸菌群がバターで 11.3%(5/44)，腸球菌群はバターで 43.1%(19/44)，脱脂粉乳で57.1%

Table 9. Bacteriological examinations of dairy products.

| Dairy products | No. of samples | No. of positive samples | | Plate count per g (ml) | |
|---|---|---|---|---|---|
| | | Enterococcus group | Coliforms | Under 50 thousand | Over 50 thousand |
| Skim milk powder | 14 | 8 | 0 | 14 | — |
| Modified milk powder | 13 | 7 | 0 | 13 | — |
| Whole milk powder | 2 | 2 | 0 | 2 | — |
| Sweetend milk powder | 2 | 2 | 0 | 2 | — |
| Dried ice cream mix | 6 | 3 | 0 | 6 | — |
| Sweetend condensed milk | 17 | 0 | 0 | 17 | — |
| Butter | 44 | 19 | 5 | 35 | 9 |
| Processed cheese | 7 | 0 | 0 | 7 | — |
| Total | 105 | 41 | 5 | 96 | 9 |

Table 10. Numbers of enterococcus group bacteria, coliform bacteria and living bacteria per g in dairy products contained enterococcus group (results in 1957).

| Sample No. | Enterococcus group bacteria (M.P.N. per g) | Plate count per g | | Sample No. | Enterococcus group bacteria (M.P.N. per g) | Plate count per g | |
|---|---|---|---|---|---|---|---|
| | | Coliform* bacteria | Living bacteria | | | Coliform* bacteria | Living bacteria |
| S 5 | 20 | 0 | 530 | B 1 | 20 | 0 | 560 |
| 〃 6 | 10 | 0 | 430 | 〃 2 | 130 | 0 | 1,200 |
| 〃 10 | 68 | 0 | 750 | 〃 3 | 45 | 0 | 75 |
| 〃 11 | 110 | 0 | 1,300 | 〃 4 | 45 | 0 | 1,400 |
| 〃 12 | 68 | 0 | 550 | 〃 10 | 68 | 0 | 120 |
| 〃 13 | 20 | 0 | 330 | 〃 14 | 20 | 0 | 800 |
| 〃 17 | 20 | 0 | 1,100 | 〃 19 | 250 | 0 | 1,400 |
| 〃 26 | 83 | 0 | 320 | 〃 20 | 10 | 0 | 35 |
| M 2 | 20 | 0 | 650 | 〃 21 | 10 | 0 | 45 |
| 〃 10 | 110 | 0 | 650 | 〃 25 | 23 | 0 | 8,000 |
| 〃 20 | 39 | 0 | 580 | 〃 26 | 23 | 0 | 240,000 |
| 〃 21 | 23 | 0 | 550 | 〃 27 | 85 | 0 | 930,000 |
| 〃 22 | 70 | 0 | 200 | 〃 28 | 110 | 300 | 99,000 |
| 〃 27 | 83 | 0 | 640 | 〃 29 | 10 | 0 | 28,000 |
| 〃 28 | 550 | 0 | 470 | 〃 31 | 400 | 30 | 5,300,000 |
| W 9 | 39 | 0 | 650 | 〃 32 | 1,700 | 9,000 | 4,800,000 |
| 〃 22 | 10 | 0 | 530 | 〃 33 | 23 | 0 | 410,000 |
| K 7 | 39 | 0 | 430 | 〃 35 | 650 | 9,300 | 4,400,000 |
| 〃 8 | 4,000 | 0 | 3,000 | 〃 36 | 55 | 100 | 78,000 |
| I 1 | 170 | 0 | 570 | | | | |
| 〃 8 | 20 | 0 | 260 | | | | |
| 〃 23 | 10 | 0 | 330 | | | | |

Notes. S : Skim milk powder, M : Modified milk powder, W : Whole milk powder, K : Sweetend milk powder, I : Dried ice cream mix, B : Butter.

* Medium : Violet Red Bile Agar (Difco).

(8/14)，調製粉乳で53.8%(7/13)，全粉乳及び加糖粉乳ではそれぞれ100%(2/2)，ミックスパウダーで50%(3/6)であった。

次に上記腸球菌群の陽性例41材料について，それらの腸球菌群数，大腸菌群数及び一般生菌数3者の出現の関係を示すとTable 10の如くである．すなわち粉乳類22例において大腸菌群は陰性であり，腸球菌群はg当り4,000が1例あったのみで残る21例は550以下の成績であった．生菌数も200～3,000で少なく，1,000以上はわずかに3例のみであった．バターにおいてB1からB25迄の10例は大メーカー製，B26からB36の9例は小メーカー製であり，腸球菌群数は大メーカー製にあっては粉乳類同様，小メーカー製にあっても2，3の例外を除いて粉乳あるいは大メーカー製同様の成績を示した．大腸菌群は大メーカー製で陰性，小メーカー製で5例が陽性であり，菌数は30～9,300で陽性5例中4例は腸球菌群数より多かった．生菌数は大メーカー製ではB25が8,000で最高を示したが，それ以外は粉乳類と同様に菌数は少なかった．これに反し小メーカー製にあっては一般に菌数は多く，2.8万～530万に分布し2万台の1例を除いて残る8例は何れも5万以上であり，5万～100万4例，100万以上3例であった．

4. 粉乳（1958年度検査分）

粉乳81材料の検査成績はTable 11に示すとおりである．

大腸菌群は全例陰性であったため本表から除外した．それに反して腸球菌群は100%陽性であり，g当りの菌数は7～1,800に分布していた．内訳は菌数100以下が42例（51.8%），110～1,000が36例（44.4%），1,100以上はわずか3例（3.7%）であり，菌数そのものは前項の粉乳の成績と比べて大差はみられない．又各工場における毎日の出現菌数はほぼ一定しているのが観察される．

生菌数は130～4,200に分布し，1,000以下66例，以上15例という良好な成績であり，腸球菌群数と同様に毎日の出現菌数はほぼ一定している．

5. 生肉A（冬季）

豚肉及び牛肉各30例計60例について行った冬季の検査成績はTable 12に示される．本表において左側豚肉No. 1～28とそれに対応する右側牛肉のNo. 51～78の各28例は28軒の小売店よりそれぞれ同時に購入されたものであり，最下段の2列すなわちNo.29，30及びNo. 79，80の4例のみは別々に4軒の店から購入された．

腸球菌群の陽性率は豚肉において66.6%（20/30），牛肉において56.6%（17/30），両者をあわせて61.6%（37/60）であったのに対し，大腸菌群のそれは豚肉で83.3%（25/30），牛肉で80%（24/30），両者あわせて81.6%（49/60）を示し，大腸菌群の陽性率は腸球菌群のそれよりかなり高かった．豚肉及び牛肉の両者における腸球菌群数はg当り0～700で，その菌数の内訳は0が23例，10以下26例，11～100が8例，110以上3例であったのに対し，大腸菌群数は0～1,300で菌数0が11例，10以下17例，11～100が23例，110以上9例という成績であった．更に同一材料において出現した両菌群の菌数の多少を比較すると，Table 13に示される様に大腸菌群数の方のより多い例が，腸球菌群数の方のより多い例よりも多数を占めた．

生菌数は豚肉で0.3万～240万，牛肉で0.2万～1,800万に分布しており，その内訳は菌数1万以下が豚肉及び牛肉でそれぞれ4，5例，1.1万～10万が共に12例，11万～100万は11，9例，110万以上3，4例と両者は同じ様な傾向で出現した．

次に豚肉と牛肉の両者を同時に購入した店28軒について，腸球菌群と大腸菌群の出現の関

Table 11. Numbers of enterococcus group bacteria and living bacteria per g in milk powder (results in 1958).

| Dairy plant | Sample | Date of manufacture | M.P.N. of enterococcus group bacteria | Plate count | Dairy plant | Sample | Date of manufacture | M.P.N. of enterococcus group bacteria | Plate count |
|---|---|---|---|---|---|---|---|---|---|
| A | S | Oct. 7 | 49 | 2,200 | C | S | Oct. 5 A | 170 | 680 |
| 〃 | 〃 | 8 A | 70 | 420 | 〃 | 〃 | 5 B | 240 | 880 |
| 〃 | 〃 | 8 B | 23 | 190 | 〃 | 〃 | 6 A | 17 | 250 |
| 〃 | 〃 | 9 A | 46 | 220 | 〃 | 〃 | 6 B | 350 | 1,000 |
| 〃 | 〃 | 9 B | 22 | 300 | 〃 | 〃 | 7 A | 350 | 680 |
| 〃 | 〃 | 10 | 33 | 270 | 〃 | 〃 | 7 B | 920 | 3,200 |
| 〃 | 〃 | 11 A | 17 | 300 | 〃 | 〃 | 8 A | 540 | 1,200 |
| 〃 | 〃 | 11 B | 33 | 480 | 〃 | 〃 | 8 B | 130 | 990 |
| 〃 | 〃 | 12 A | 46 | 230 | 〃 | 〃 | 9 A | 79 | 210 |
| 〃 | 〃 | 12 B | 130 | 200 | 〃 | 〃 | 9 B | 920 | 1,500 |
| 〃 | 〃 | 13 A | 79 | 350 | 〃 | 〃 | 10 A | 110 | 370 |
| 〃 | 〃 | 13 B | 46 | 350 | 〃 | 〃 | 10 B | 280 | 1,100 |
| 〃 | 〃 | 14 A | 79 | 310 | D | W | Oct. 5 | 130 | 750 |
| 〃 | 〃 | 14 B | 240 | 690 | 〃 | 〃 | 6 | 23 | 1,200 |
| 〃 | 〃 | 15 A | 46 | 290 | 〃 | 〃 | 7 | 79 | 230 |
| 〃 | 〃 | 15 B | 7 | 320 | 〃 | 〃 | 9 | 130 | 250 |
| 〃 | 〃 | 16 A | 49 | 190 | 〃 | 〃 | 10 | 130 | 320 |
| 〃 | 〃 | 16 B | 49 | 250 | 〃 | S | 12 | 33 | 2,200 |
| 〃 | 〃 | 17 A | 79 | 440 | 〃 | 〃 | 14 | 130 | 440 |
| 〃 | 〃 | 17 B | 79 | 470 | 〃 | 〃 | 15 | 23 | 450 |
| 〃 | 〃 | 18 A | 17 | 190 | 〃 | 〃 | 16 | 240 | 350 |
| 〃 | 〃 | 18 B | 11 | 700 | 〃 | 〃 | 18 | 220 | 450 |
| 〃 | 〃 | 19 A | 23 | 360 | 〃 | W | 19 | 79 | 500 |
| 〃 | 〃 | 19 B | 23 | 140 | 〃 | 〃 | 20 | 110 | 300 |
| B | 〃 | Oct. 16 | 1,600 | 2,100 | 〃 | 〃 | 21 | 79 | 190 |
| 〃 | 〃 | 17 | 1,600 | 1,900 | 〃 | 〃 | 22 | 33 | 300 |
| 〃 | 〃 | 18 | 920 | 1,200 | 〃 | 〃 | 23 | 170 | 290 |
| 〃 | 〃 | 19 | 920 | 940 | 〃 | 〃 | 24 | 130 | 250 |
| 〃 | 〃 | 20 | 540 | 760 | 〃 | 〃 | 25 | 350 | 440 |
| 〃 | 〃 | 21 | 1,800 | 2,600 | 〃 | S | 29 | 64 | 250 |
| 〃 | 〃 | 22 | 110 | 570 | 〃 | 〃 | 30 | 33 | 3,500 |
| 〃 | 〃 | 23 | 350 | 370 | 〃 | 〃 | 31 | 49 | 230 |
| 〃 | 〃 | 24 | 350 | 590 | E | 〃 | Oct. 1 | 49 | 1,300 |
| 〃 | 〃 | 25 | 540 | 700 | 〃 | 〃 | 2 | 49 | 240 |
| 〃 | 〃 | 26 | 79 | 380 | 〃 | 〃 | 4 | 33 | 210 |
| 〃 | 〃 | 27 | 350 | 730 | 〃 | 〃 | 5 | 17 | 130 |
| C | 〃 | Oct. 2 | 220 | 570 | 〃 | 〃 | 6 | 130 | 300 |
| 〃 | 〃 | 3 A | 110 | 680 | 〃 | 〃 | 7 | 79 | 410 |
| 〃 | 〃 | 3 B | 540 | 4,200 | 〃 | 〃 | 8 | 79 | 250 |
| 〃 | 〃 | 4 A | 170 | 620 | 〃 | 〃 | 9 | 31 | 240 |
| 〃 | 〃 | 4 B | 220 | 2,200 | | | | | |

Notes. Coliforms were all negative in these samples.
S : Skim milk powder, W: Whole milk powder.

Table 12. Numbers of enterococcus group bacteria, coliform bacteria and living bacteria per g in raw meat from meat-shops (in Winter).

| Sample No. | Pork | | | Sample No. | Beef | | |
|---|---|---|---|---|---|---|---|
| | M.P.N. per g | | Plate count/g (Unit : 10 thousand) | | M.P.N. per g | | Plate count/g (Unit : 10 thousand) |
| | Enterococcus group bacteria | Coliform bacteria | | | Enterococcus group bacteria | Coliform bacteria | |
| 1 | 350 | 33 | 25.0 | 51 | 130 | 4.5 | 4.5 |
| 2 | 33 | >7.8 | 7.4 | 52 | 2 | 0 | 1.0 |
| 3 | 22 | >6.8 | 1.2 | 53 | 23 | >11 | 15.0 |
| 4 | 13 | 1,300 | >50.0 | 54 | 7.8 | >45 | 5.6 |
| 5 | 13 | 4,900 | 240.0 | 55 | 11 | 23 | >20.0 |
| 6 | 7.8 | >13 | 22.0 | 56 | 11 | 0 | >120.0 |
| 7 | 6.8 | 79 | 13.0 | 57 | 2 | 70 | 33.0 |
| 8 | 4.5 | >17 | >5.0 | 58 | 4.5 | >13 | 3.1 |
| 9 | 4.5 | >11 | 9.9 | 59 | 7.8 | 170 | 380.0 |
| 10 | 2 | 0 | 31.0 | 60 | 7.8 | >4.5 | 2.8 |
| 11 | 2 | 0 | 6.0 | 61 | 700 | 43 | 1,800.0 |
| 12 | 2 | 0 | 33.0 | 62 | 4.5 | 79 | 180.0 |
| 13 | 4.5 | 790 | 8.0 | 63 | 0 | >7.8 | 5.0 |
| 14 | 4.5 | >2 | 2.8 | 64 | 0 | 0 | 5.4 |
| 15 | 2 | 95 | 200.0 | 65 | 0 | 33 | 60.0 |
| 16 | 2 | >7.8 | 0.3 | 66 | 0 | >7.8 | 0.2 |
| 17 | 2 | 1.8 | 8.5 | 67 | 0 | >6.1 | 8.6 |
| 18 | 2 | >110 | 6.1 | 68 | 0 | >9.3 | 32.0 |
| 19 | 1.8 | >21 | 60.0 | 69 | 0 | 23 | 3.7 |
| 20 | 0 | >17 | 9.8 | 70 | 23 | 0 | 3.3 |
| 21 | 0 | >4.5 | 0.3 | 71 | 7.8 | >140 | 1.1 |
| 22 | 0 | 130 | 0.7 | 72 | 2 | 110 | 9.3 |
| 23 | 0 | >2 | 6.2 | 73 | 2 | 0 | 0.4 |
| 24 | 0 | >20 | 52.0 | 74 | 0 | >14 | 12.0 |
| 25 | 0 | >11 | 6.4 | 75 | 0 | >2 | >60.0 |
| 26 | 0 | >6.8 | 44.0 | 76 | 0 | 110 | >55.0 |
| 27 | 0 | >4.5 | 0.9 | 77 | 0 | >93 | 100.0 |
| 28 | 0 | 14 | >140.0 | 78 | 0 | >13 | 2.3 |
| 29 | 4.5 | 0 | 100.0 | 79 | 0 | 0 | 0.9 |
| 30 | 0 | 0 | 29.0 | 80 | 2 | >2 | 0.8 |

Table 13. Comparison of numbers of enterococcus group bacteria and coliform bacteria in raw meat from meat-shops (in Winter).

| Appearances | No. of samples | |
|---|---|---|
| | Pork | Beef |
| Enterococcus group bacteria less than coliform bacteria | 20 | 20 |
| 〃 〃 〃 more 〃 〃 〃 | 6 | 6 |
| Equal or not compared | 4 | 4 |

係をみるとTable 14の如く，全部陽性が25%(7/28)，全部陰性は0であった．すなわち小売店の生肉はこれら菌群の汚染を受ける機会の多い事が判明する．

Table 14. Relationship of appearance of enterococcus group and coliforms in pork and beef purchased in pair from 28 meat-shops.

| Pork | | Beef | | No. of shops |
|---|---|---|---|---|
| Enterococcus group | Coliforms | Enterococcus group | Coliforms | |
| + | + | + | + | 7 |
| − | − | − | − | 0 |
| + | • | + | • | 12 |
| • | + | • | + | 20 |
| + | + | • | • | 16 |
| • | • | + | + | 12 |
| + | − | • | • | 3 |
| • | • | + | − | 4 |
| − | + | • | • | 9 |
| • | • | − | + | 11 |
| − | − | • | • | 0 |
| • | • | − | − | 1 |

豚肉，牛肉の両者共腸球菌群陽性は42.8% (12/28)，大腸菌群陽性は71.4% (20/28)であった。豚肉において両菌群共陽性は57.1% (16/28)，牛肉のそれは42.8% (12/28)であり，豚肉において腸球菌群のみ陽性は10.7% (3/28)，大腸菌群のみ陽性は32.1% (9/28)，牛肉において腸球菌群のみ陽性は14.2%(4/28)，大腸菌群のみ陽性は39.2%(11/28)を示し，豚肉と牛肉の汚染度を比較すると豚肉の方が両菌群共に牛肉より汚染されている率が高かった．

生肉B（夏季）

豚肉，牛肉各20例計40例の夏季における検査成績はTable 15に示すとおりである．本例も冬季同様豚肉，牛肉の両者を同一店から購入した例は表左側のNo. 31～48とそれに対応する牛肉のNo.81～98の18軒分である．

腸球菌群並びに大腸菌群は冬季と異り両者共100%陽性であった．又菌数においても冬季に比べて著しく多いのが注目される．すなわち両者の生肉における腸球菌群数はg当り，11～160,000に分布しており，その内訳は100以下9例，110～1,000が12例，1,100～1万が16例，1.1万以上3例であった．大腸菌群数は＞7～＞49,000に分布し，大略100以下5例，110～1,000が10例，1,100～1万が14例，1.1万以上11例であり，個々の材料について両菌群の出現菌数の多少を比較すると，Table 16に示される様に，冬季同様大腸菌群数の方が腸球菌群数よりも多い方の例が，逆の例より多数を占めた．

生菌数は冬季のそれより1桁多く，8万～＞2,500万に分布し，その内訳は10万以下豚肉で0，牛肉で1例，11万～100万はそれぞれ9，8例，110万～1,000万もそれぞれ9，8例，1,100万以上は2，3例で両者の間に同じ様な傾向で出現するのは冬季同様であるが，出現菌数は上述の如く冬季の約10倍の成績であった．

次に豚肉及び牛肉の両者を同時に購入した18軒についての冬季同様の比較は何れも100%陽性のため検討出来なかった．すなわち夏季の小売店における生肉は全例がこれら菌群の汚

Table 15. Numbers of enterococcus group bacteria, coliform bacteria and living bacteria per g in raw meat from meat-shops (in Summer).

| Sample No. | Pork | | | Sample No. | Beef | | |
|---|---|---|---|---|---|---|---|
| | M.P.N. per g | | Plate count/g (Unit : 10 thousand) | | M.P.N. per g | | Plate count/g (Unit : 10 thousand) |
| | Enterococcus group bacteria | Coliform bacteria | | | Enterococcus group bacteria | Coliform bacteria | |
| 31 | 160,000 | >24,000 | >2,500 | 81 | 1,100 | >24,000 | 1,500 |
| 32 | 54,000 | >24,000 | >2,400 | 82 | 4,900 | >7,900 | 500 |
| 33 | 54,000 | >33,000 | 65 | 83 | 11 | >11 | 40 |
| 34 | 7,900 | >7,900 | 900 | 84 | 1,800 | >4,900 | 1,600 |
| 35 | 3,500 | >70 | 44 | 85 | 2,400 | >410 | 47 |
| 36 | 2,400 | >180 | 16 | 86 | 34 | 700 | 44 |
| 37 | 1,300 | <220 | 18 | 87 | 4,900 | 13,000 | 470 |
| 38 | 1,100 | >24,000 | >250 | 88 | 5,400 | >24,000 | >2,500 |
| 39 | 790 | >9,500 | 40 | 89 | 2,200 | >1,700 | 57 |
| 40 | 790 | >7 | 23 | 90 | 3,300 | 240 | 86 |
| 41 | 790 | >7,900 | 520 | 91 | 700 | >49,000 | 280 |
| 42 | 490 | >4,900 | 130 | 92 | 240 | >1,400 | 84 |
| 43 | 490 | 790 | 150 | 93 | 1,400 | 330 | 220 |
| 44 | 460 | >7,900 | >93 | 94 | 79 | >3,300 | 110 |
| 45 | 240 | 2,400 | >190 | 95 | 2,400 | >13,000 | >300 |
| 46 | 22 | 1,300 | >100 | 96 | 330 | >33,000 | 72 |
| 47 | 20 | 130 | >120 | 97 | 79 | 2,200 | 180 |
| 48 | 13 | 79 | 43 | 98 | 33 | <220 | 8 |
| 49 | 130 | >46 | >110 | 99 | 240 | 790 | >65 |
| 50 | 13 | 1,300 | 42 | 100 | >1,800 | >49,000 | >380 |

Table 16. Comparison of numbers of enterococcus group bacteria and coliform bacteria in raw meat from meat-shops (in Summer).

| Appearances | No. of samples | |
|---|---|---|
| | Pork | Beef |
| Enterococcus group bacteria less than coliform bacteria | 12 | 14 |
| 〃 〃 〃 more 〃 〃 〃 | 1 | 2 |
| Equal or not compared | 7 | 4 |

染を受けている事を知った.

6. 肉 製 品

Table 17 に示すようにドメスチックソーセージ7例 (No. 1～7), ドライソーセージ13例 (No. 8～20), ハム17例 (No. 21～35) 及びベーコン2例 (No. 38, 39 但し後者は鯨製品) の計39材料について検査を行った.

腸球菌群の陽性率は23%(9/39)であり, 菌数の分布はg当り10以下4例, 11～100は2例, 100台が2例で1例のみが12,000とかけ離れて出現した. ソーセージのうちサラミソーセージのみは2例共, 又スライスされた真空包装のスモークハム及びベーコンの4例では3例が

Table 17. Numbers of enterococcus group bacteria, coliform bacteria and living bacteria per g in meat products.

| Sample No. | Kinds | M.P.N. per g | | Plate count per g | Sample No. | Kinds | M.P.N. per g | | Plate count per g |
|---|---|---|---|---|---|---|---|---|---|
| | | Enterococcus group bacteria | Coliform bacteria | | | | Enterococcus group bacteria | Coliform bacteria | |
| 1 | Sausage | 0 | <2 | 4,500 | 21 | Lachs ham | 0 | <2 | 2,800,000 |
| 2 | 〃 | 0 | 0 | 17,000 | 22 | 〃 〃 | 120 | <6 | 1,000,000 |
| 3 | Bologna style sausage | 0 | 0 | 350 | 23 | 〃 〃 | 0 | 0 | 1,500 |
| 4 | Frankfurt 〃 〃 | 0 | 0 | 5,000 | 24 | 〃 〃 | 0 | <2 | 22,000 |
| 5 | Vienna 〃 〃 | 0 | 0 | 30,000 | 25 | 〃 〃 | 150 | <2 | 150,000 |
| 6 | 〃 〃 〃 | 0 | 0 | 8,800 | 26 | Pressed ham (Round form) | 6 | 0 | 3,300 |
| 7 | Cheese sausage | 0 | <2 | 3,000,000 | 27 | 〃 〃 ( 〃 〃 ) | 0 | 0 | 33,000 |
| 8 | Russian style sausage | 0 | 0 | 5,000 | 28 | 〃 〃 ( 〃 〃 ) | 2 | <2 | >7,500,000 |
| 9 | 〃 〃 〃 | 0 | 0 | 230 | 29 | 〃 〃 ( 〃 〃 ) | 0 | 0 | 3,000 |
| 10 | 〃 〃 〃 | 0 | 0 | 40 | 30 | 〃 〃 ( 〃 〃 ) | 0 | 0 | 2,500 |
| 11 | 〃 〃 〃 | 0 | 0 | 4,000 | 31 | 〃 〃 (Cubic 〃 ) | 0 | 0 | 1,300,000 |
| 12 | Krakawa 〃 〃 | 0 | 0 | >250,000 | 32 | 〃 〃 ( 〃 〃 ) | 0 | 0 | 330 |
| 13 | 〃 〃 〃 | 0 | 0 | 600 | 33 | 〃 〃 ( 〃 〃 ) | 0 | <4 | >25,000 |
| 14 | 〃 〃 〃 | 0 | 0 | 5,500 | 34 | 〃 〃 ( 〃 〃 ) | 0 | <2 | 9,500 |
| 15 | 〃 〃 〃 | 0 | <2 | 7,300 | 35 | 〃 〃 ( 〃 〃 ) | 0 | 0 | 16,000 |
| 16 | 〃 〃 〃 | 0 | 0 | 1,300,000 | 36 | Sliced smoked ham | 1 | 0 | >100,000,000 |
| 17 | 〃 〃 〃 | 0 | <3 | 600 | 37 | 〃 〃 〃 | 0 | 0 | >130,000,000 |
| 18 | Salami 〃 〃 | 17 | 0 | 2,000 | 38 | 〃 bacon | 3 | 0 | >44,000,000 |
| 19 | 〃 〃 〃 | 11 | 0 | 850 | 39 | Sliced bacon style of whale meat | 12,000 | >120 | >7,500,000 |
| 20 | Pepperoni 〃 〃 | 0 | 0 | 500 | | | | | |

陽性であった．なお最高の腸球菌群数（12,000）を示したのはこのうちの鯨製ベーコンである．大腸菌群の陽性率は30.7%(12/39)で，腸球菌群陽性率よりやや高く，検出頻度は製品の種類別にみるとソーセージ類に比べてハムに多く，又前記腸球菌群数の最も多かった鯨製ベーコンでは本菌群も最高に検出された．菌数の分布は100台が1例のみで残りの11例は10以下であった．両菌群共陽性は4例にみられたが，それらの菌数の比較では何れも腸球菌群の方が多かった．

生菌数は40～＞1.3億に迄分布し，1万以下が53.8%(21/39)で約半数を占めるが，1.1万～10万が6例，11万～100万3例，110万～1,000万6例，1,100万以上3例であり，特にスライスされたもの（No.36～39）に菌数の多いのが注目される．なおNo.38のベーコン1例を除く他は何れも加熱処理する事なく，そのままの状態で食される可能性の多いものだけに，100万以上が25.6%(10/39)もあるのは注目すべき点である．

次に3者の菌の出現状態をメーカー別にみるとTable 18の如く，腸球菌群及び大腸菌群はそれぞれ7メーカーにみられた．乳及び乳製品には生菌数の規格が定められているが，肉製品にはそのような規格がみられない．しかし乳及び乳製品にならって一応5万を基準として分類すると，5万以下27例，5万以上12例(30.7%)であった．また生菌数は5万以下で大腸菌群も陰性の製品を一応合格とすると，表の右端に示されるように計21例(53.8%)が相当する．更にこの規格をおし進めて，腸球菌群もあわせて陰性を必要条件とすると，18例(46.1%)が合格となりその率は少しく下がる．生菌数5万以上あるいは大腸菌群陽性あるいは腸球菌群陽性といった例はメーカーを問わず検出されているのが認められる．

Table 18. Relationship between appearance of enterococcus group, coliforms and number of living bacteria in meat products manufactured by different makers.

| Maker | No. of Samples | No. of positive samples | | Plate count per g | | Favourable* | |
|---|---|---|---|---|---|---|---|
| | | Enterococcus group | Coliforms | Under 50 thousand | Over 50 thousand | No. of samples | % |
| A | 10 | 1 | 2 | 9 | 1 | 8 | 80.0 |
| B | 6 | 1 | 4 | 5 | 1 | 2 | 33.3 |
| C | 5 | 1 | 1 | 4 | 1 | 4 | 80.0 |
| D | 5 | • | 1 | 4 | 1 | 3 | 60.0 |
| E | 5 | 2 | 1 | 2 | 3 | 2 | 40.0 |
| F | 5 | 2 | 2 | 3 | 2 | 2 | 40.0 |
| G | 2 | 1 | • | • | 2 | • | 0 |
| H | 1 | 1 | 1 | • | 1 | • | 0 |
| Total | 39 | 9 | 12 | 27 | 12 | 21 | 53.8 |

*：The sample which coliforms was negative and bacterial count was under 50 thousand per g, was supposed as favourable.

## IV 小　　括

各種畜産食品における腸球菌群，大腸菌群及び一般生菌数の出現状況をまとめるとTable 19の如くである．なお1957年度検査分の各種乳製品は材料の種類により，出現菌の傾向に差がみられるので，煉乳とプロセスチーズ，バター及び粉乳の3項目に分けて示した．非加熱材料においては，乳房直接採取乳は別としてプラント持込乳及び夏季の生肉材料では，腸

球菌群ならびに大腸菌群とも100%陽性を示し，冬季の生肉材料ではそれぞれ62%及び82%の陽性率であった．これら材料はまた生菌数も多く，大半が10万以上から更に1,000万以上にまで分布していた．

Table 19. Relationship between positive case of enterococcus group or coliforms and number of living bacteria in milk, meat and their products.

| Division | No. of samples | No. of positive samples | | Range of plate count per g (ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Enterococcus group | Coliforms | Under 50 thousand | 51～100 thousand | 110 thousand～1 million | 1.1～4 million | 4.1～10 million | Over 11 million |
| Milk from udder directly | 200 | 6 | | | | | | | |
| Raw milk from plant | 50 | 50 | 50 | • | 1 | 25 | 18 | 2 | 4 |
| Raw meat (in Winter) | 60 | 37 | 49 | 19 | 14 | 20 | 6 | • | 1 |
| 〃 〃 (in Summer) | 40 | 40 | 40 | • | 1 | 17 | 14 | 3 | 5 |
| Sweetend condensed milk and processed cheese (in 1957) | 24 | • | • | 24 | • | • | • | • | • |
| Butter (in 1957) | 44 | 19 | 5 | 35 | 2 | 3 | • | 4 | • |
| Powdered milk (in 1957) | 37 | 22 | • | 22 | • | • | • | • | • |
| 〃 〃 (in 1958) | 81 | 81 | • | 81 | • | • | • | • | • |
| Meat products | 39 | 9 | 12 | 27 | • | 3 | 4 | 2 | 3 |

加熱材料たる製品において，煉乳及びプロセスチーズでは両菌群ともすべて陰性，生菌数も5万以下であったが，バターにおいて腸球菌群の陽性率は43.1%，粉乳では1957年度検査分59.4%，1958年度検査分100%，肉製品では23.0%であり，大腸菌群はバターで11.3%，肉製品で30.7%の陽性率であった．生菌数は粉乳において両年度とも5万以下であったが，バター及び肉製品では1,000万あるいはそれ以上にまで分布しているのがみられた．

非加熱材料たる持込乳及び夏季の生肉材料では腸球菌群及び大腸菌群は100%陽性であり，両菌群の陽性率を比較できないが，冬季の生肉では腸球菌群陽性率より大腸菌群のそれが大であった．また個々の材料における出現菌数の比較では，生乳，生肉を問わずすべて大腸菌群数が腸球菌群数より多い方の例が逆の例よりも多数を占めていた．

一方加熱材料とみられる乳肉製品においてバターでは腸球菌群の陽性率が大腸菌群のそれより高く，粉乳では腸球菌群のみが陽性であった．しかるに肉製品のあるものはその製造上の工程からみて完全に加熱材料とはいい難いものもあり，また製品の形態からみても乳製品とは同列に論じられないが小メーカー製のバターの成績と比較的似た成績を示した．但し大腸菌群陽性率は腸球菌群のそれより高くバターと逆の成績を示した．

大腸菌群陽性のバター及び肉製品について例数は少ないが両菌群の出現菌数を比較すると，バターでは非加熱材料同様に大腸菌群の方が高い成績を示した．肉製品では両者とも陽性がわずか3例で比較が困難であるが，本3例ではすべて腸球菌群の方が多く，その他の例においても一般に腸球菌群数の方が多い傾向にあった．

製品における生菌数と両菌群の出現は，生菌数5万以上の例が存したのは大腸菌群陽性例がみられたバター及び肉製品においてのみであったこと，バターにおける大腸菌群陽性の5例はすべて生菌数も5万以上であり，また生菌数5万以上の9例中8例は腸球菌群が陽性であったこと，肉製品においては腸球菌群陽性の9例中5例，大腸菌群陽性の12例中6例が生菌数も5万以上の材料であったこと等，種々の点において3者の成績の関係深いことが観察された．

次に生乳から乳製品に至る腸球菌群の出現状況をみると，乳房から直接採取した乳ではわずか3％が陽性であり，しかもその出現菌数はほとんどがml当り小数点以下の成績を示していることから，この段階における汚染は非常に低いものと考えられる．しかしプラントに持込まれた原料乳の段階では100％陽性となり，更に製品の段階において1957年検査の煉乳，プロセスチーズは陰性，バターで43％，粉乳で59％，1958年検査の粉乳では100％陽性という成績を示した．但し粉乳の両年度における検査方法は既に述べたとおり少しく異っているため，前年度における陽性率は後年度の方法を用いていたならばあるいはもう少し高まったかも知れない．また以上の検査材料はすべて一貫した材料でないため強くいい切るのは難かしいが，何れにせよ搾乳から製品に至る過程において腸球菌群は乳房直接採取乳ではほぼ陰性であるが，プラント持込乳では100％の汚染率を示すに至り，次いで製品の段階においても数そのものは減少こそすれ依然として残存する．特に粉乳において残存する率が高いといえそうである．

以上要するにプラント持込乳，生肉等の生材料において，腸球菌群，大腸菌群及び一般生菌数三者の出現状況の間にはそれぞれ関連性のあることが認められる．またこれら生材料からの加工品においても，現段階の肉製品並びに小メーカー製のバターでは，生材料におけると同様の傾向が認められるが，粉乳においては大腸菌群陰性，生菌数もg当り5万はもとより5,000以下の現況であるにも拘らず，腸球菌群のみが検出されることは本菌群の汚染指標菌としての意義を深からしめるものである．

## (IV) 人及び各種動物の糞便における腸球菌群の分布

前章においては各種畜産食品における腸球菌群と大腸菌群の陽性率並びにそれらの出現菌数を検査して両菌群による汚染状態を比較したが，本章においては第1次汚染源となる人及び各種動物の糞便材料について前章同様の検索を行い比較検討した．

また乳房から直接採取した乳ではほとんど陰性の腸球菌群がプラント持込原料乳及び粉乳の段階において100％陽性の例がみられたことから，特に人及びウシの糞便を重点的に取上げて腸球菌群のみの検査を行い汚染源としての糞便における分布を調査した．

### I 検査材料及び方法

検査材料は1957年10月から1958年7月に至る期間と，1960年10月から12月の間に集めたものであり，前者はわれわれと比較的接触が多いと思われる9種の成獣畜(ウシ，ウマ，ヒツジ，ブタ，イヌ，ネコ，ネズミ，ウサギ，ニワトリ)，生後2週間以内の初生児及び成人の糞便各5例計55例である．後者は児童及び成人50例，成牛30例の糞便であり総計135例である．前者の初生児は札幌市内の1病院で哺育中の乳児，成人は教室員であり，各種動物は北海道大学付属農場飼育の家畜，教室飼育の実験動物及び動物舎において捕えられたネズミである．後者の人は教室員とその家族，北大獣医学部学生及び小学児童であり，児童の糞便は札幌市

の1保健所より提供を受けた．ウシは付属農場及びその他5戸の酪農家飼育のものである．

以上の糞便材料1gに滅菌生理食塩水9mlを加えてよく磨砕し，10倍稀釈液を作って以後の実験に供した．すなわち前者の材料は腸球菌群並びに大腸菌群の両方を，後者の材料は腸球菌群のみについて検査を行った．腸球菌群の検査方法は前章で述べたとおりであるが，後者の材料については(II)章の成績に鑑み培地No. 2を使用した．大腸菌群の検査は前章と同様の方法で行った．

## II 検 査 成 績

### 1. 人及び各種動物の糞便

各種動物9種と初生児及び成人の糞便55例について腸球菌群と大腸菌群の陽性率ならびに菌数の分布状態を調査して比較検討した．個々の材料における両菌群の出現菌数の成績は

Table 20. M.P.N. of enterococcus group bacteria and coliform bacteria per g in human and various animal feces.

| Sample | | M.P.N. per g | | Sample | | M.P.N. per g | |
|---|---|---|---|---|---|---|---|
| No. | Origin | Enterococcus group bacteria | Coliform bacteria | No. | Origin | Enterococcus group bacteria | Coliform bacteria |
| 1 | Cattle | <12,000 | 1,800 | 31 | Fowl | 65,000,000 | 13,000,000 |
| 2 | 〃 | <12,000 | 33,000 | 32 | 〃 | 65,000,000 | 1,700,000 |
| 3 | 〃 | <12,000 | 3,300,000 | 33 | 〃 | 7,000,000 | 4,500,000 |
| 4 | 〃 | 650 | 140,000 | 34 | 〃 | 8,500,000 | 7,900,000 |
| 5 | 〃 | 1,100 | 170,000 | 35 | 〃 | 2,500,000 | 13,000 |
| 6 | Horse | 100,000 | <23,000 | 36 | Rat | 170,000,000 | 7,000,000 |
| 7 | 〃 | 100 | 45,000 | 37 | 〃 | 65,000,000 | 13,000,000 |
| 8 | 〃 | 25,000 | 24,000 | 38 | 〃 | 650,000 | 49,000 |
| 9 | 〃 | 5,500 | 3,300,000 | 39 | 〃 | 650,000 | 1,300,000 |
| 10 | 〃 | 170,000 | 240,000 | 40 | 〃 | 850,000 | 130,000 |
| 11 | Sheep | 4,000,000 | 13,000,000 | 41 | Rabbit | 400,000 | >24,000,000 |
| 12 | 〃 | 1,200,000 | 17,000,000 | 42 | 〃 | 12,000 | <23,000 |
| 13 | 〃 | 2,500,000 | 22,000,000 | 43 | 〃 | 38,000 | <23 |
| 14 | 〃 | 1,700,000 | 79,000,000 | 44 | 〃 | 17,000 | 790,000 |
| 15 | 〃 | 350,000 | 4,900,000 | 45 | 〃 | <12 | <23 |
| 16 | Hog | 4,000,000 | 3,300,000 | 46 | Human (Suckling baby) | >12,000,000 | >240,000 |
| 17 | 〃 | 100,000 | 230,000 | | | | |
| 18 | 〃 | 2,500,000 | 49,000,000 | 47 | 〃 (〃 〃) | >12,000,000 | >240,000 |
| 19 | 〃 | 55,000 | 330,000 | 48 | 〃 (〃 〃) | 100,000 | 5,400,000 |
| 20 | 〃 | 110,000 | 1,300,000 | 49 | 〃 (〃 〃) | 2,700,000,000 | <23,000 |
| 21 | Dog | 170,000,000 | >240,000,000 | 50 | 〃 (〃 〃) | 8,500,000 | 110,000,000 |
| 22 | 〃 | 17,000,000 | >240,000,000 | 51 | 〃 (Adult) | 230,000 | 490,000 |
| 23 | 〃 | 11,000,000 | 330,000,000 | 52 | 〃 ( 〃 ) | 700,000,000 | 7,000,000 |
| 24 | 〃 | 180,000,000 | 490,000,000 | 53 | 〃 ( 〃 ) | <120,000 | 24,000,000 |
| 25 | 〃 | 25,000,000 | 4,900,000 | 54 | 〃 ( 〃 ) | 850 | 79,000 |
| 26 | Cat | 100,000 | 78,000 | 55 | 〃 ( 〃 ) | 230 | 49,000,000 |
| 27 | 〃 | 170,000 | <23 | | | | |
| 28 | 〃 | 1,000 | 330 | | | | |
| 29 | 〃 | 17,000,000 | 1,300,000,000 | | | | |
| 30 | 〃 | 120,000 | 11,000,000 | | | | |

Table 21. Range of M.P.N. of enterococcus group bacteria and coliform bacteria per g in every 5 samples of human and various animal feces.

| Origin | Enterococcus group bacteria | Coliform bacteria |
|---|---|---|
| Cattle | $10^2$～$10^4$ | $10^3$～$10^6$ |
| Horse | $10^2$～$10^5$ | $10^4$～$10^8$ |
| Sheep | $10^5$～$10^6$ | $10^8$～$10^7$ |
| Hog | $10^4$～$10^6$ | $10^5$～$10^7$ |
| Dog | $10^7$～$10^8$ | $10^6$～$10^8$ |
| Cat | $10^3$～$10^7$ | 10 ～$10^9$ |
| Fowl | $10^6$～$10^7$ | $10^4$～$10^7$ |
| Rat | $10^5$～$10^8$ | $10^4$～$10^7$ |
| Rabbit | 10 ～$10^5$ | 10 ～$10^7$ |
| Human (Suckling baby) | $10^5$～$10^9$ | $10^4$～$10^8$ |
| 〃 (Adult) | $10^2$～$10^8$ | $10^4$～$10^7$ |

Table 20 に，各種 5 例毎にまとめた成績は Table 21 に示す．

腸球菌群及び大腸菌群は両者とも 100%陽性であった．両菌群の出現菌数は 動物の種類，あるいは更に個体間においても差がみられる．そのうち個体差の比較的少なかったのは，腸球菌群ではヒツジ，イヌ，ニワトリ，ブタであり，大腸菌群ではヒツジ，ウマ，ブタ，イヌ等である．これに反して個体差の著しいものは腸球菌群では成人，ネコ，ウサギ，初生児であり，大腸菌群ではネコ，ウサギ，初生児であった．

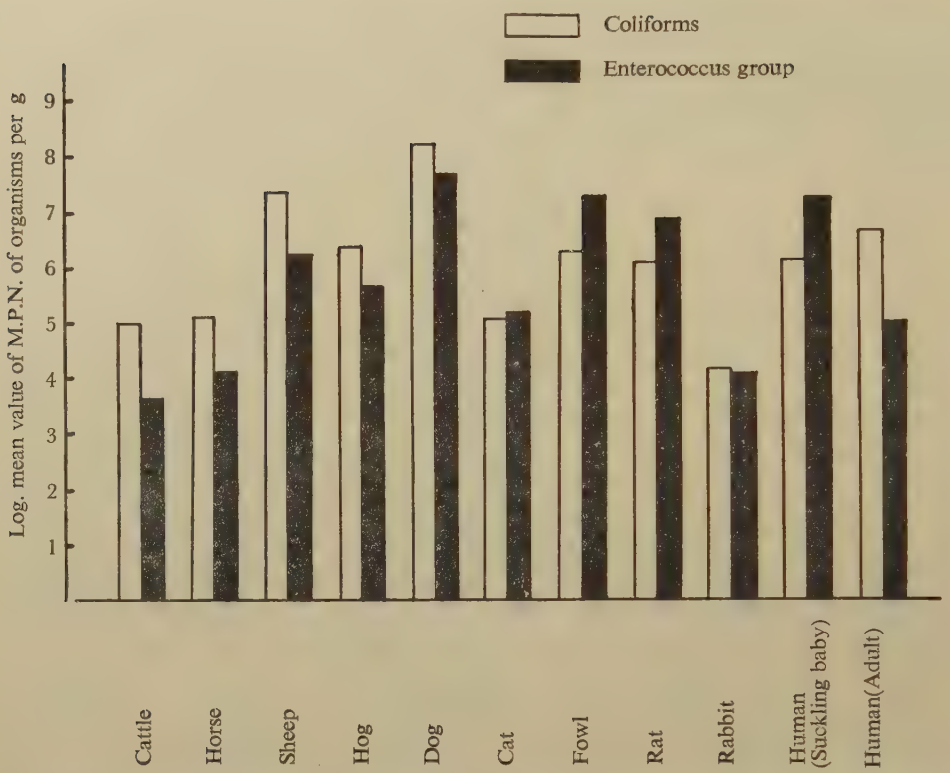
腸球菌群の g 当り菌数の最低はウサギの＜12であり，最高は初生児の27億である．その分布は 1 万以下 8 例，1.1万～10万が12例，11万～100万が 11例，110万～1,000万 10例，1,100万～1 億 9 例，1.1億以上 5 例であった．

一方大腸菌群数は最低がネコ及びウサギの＜23であり，最高はネコの13億であった．その分布は1万以下5例，1.1万～10万10例，11万～100万10例，110万～1,000万13例，1,100万～1億11例，1.1億以上 6 例であった．両菌群の出現菌数を各例毎に比較すると，Table 22 に示すように大腸菌群数のより多い例と腸球菌群数のより多い例との割合は大略 3：2 となった．

Table 22. Comparison of numbers of enterococcus group bacteria and coliform bacteria in human and various animal feces.

| Appearances | No. of samples |
|---|---|
| Enterococcus group bacteria less than coliform bacteria | 31 |
| 〃 〃 〃 more 〃 〃 〃 | 21 |
| Not compared | 3 |

次に人及び各種動物 5 例毎の両菌群の菌数の対数平均値をとり，ヒストグラムにして示したのが Text-fig. 1 である，これについて出現菌数を比較すると，腸球菌群数の平均値はイヌが一番多く，ついでニワトリ，初生児，ネズミ，ヒツジの順であり，ウシが最も少ない．大腸菌群数は同じくイヌが最も多く，ついでヒツジ，成人，ブタ，ニワトリの順であった．また両菌群の出現菌数の比較では，腸球菌群の方が大腸菌群より多いのはニワトリ，ネズミ，初生児であり，大腸菌群が腸球菌群より多いのはウシ，ウマ，ヒツジ，ブタ，イヌ及び成人




Text-fig. 1. Logarithmic mean value of M.P.N. of enterococcus group bacteria and coliform bacteria in human and various animal feces.

Table 23. Number of enterococcus group bacteria per g in bovine feces.

| Sample No. | Stall | M.P.N. of enterococcus group bacteria | Sample No. | Stall | M.P.N. of enterococcus group bacteria |
|---|---|---|---|---|---|
| 1 | A | 49 | 16 | C | 330 |
| 2 | 〃 | 49 | 17 | 〃 | 460 |
| 3 | 〃 | 79 | 18 | 〃 | 700 |
| 4 | 〃 | 79 | 19 | 〃 | 1,100 |
| 5 | 〃 | 110 | 20 | 〃 | 1,700 |
| 6 | 〃 | 240 | 21 | 〃 | 5,400 |
| 7 | 〃 | 330 | 22 | D | 70 |
| 8 | 〃 | 330 | 23 | 〃 | 110 |
| 9 | 〃 | 490 | 24 | 〃 | 1,700 |
| 10 | 〃 | 17,000 | 25 | 〃 | 13,000 |
| 11 | B | 45 | 26 | E | 23 |
| 12 | 〃 | 330 | 27 | 〃 | 45 |
| 13 | 〃 | 460 | 28 | 〃 | 170 |
| 14 | 〃 | 4,900 | 29 | 〃 | 240 |
| 15 | 〃 | 54,000 | 30 | F | 1,700 |

であった．ネコ及びウサギでは両菌群の菌数の差が少なく，また前述の如く個体間の差の著しい材料であったため，この成績から両菌群数の差を明らかにすることは困難である．

以上の如く動物の種類別，個体別にみても大腸菌群数は腸球菌群数に比べて多い例が多数を占め，総体的にみても大腸菌群数の方の多いことが認められた．

2. 再びウシの糞便について

温血動物の糞便に大腸菌群が常在することは以前から認められており，菌数の分布に関しても多数の報告[23)61)64)66)67)82)84)]がなされているので，今回の材料については大腸菌群数の検査は省略した．6牛舎由来のウシ糞便30例についての腸球菌群の検査成績は Table 23 に示される．

本表にみられるように腸球菌群は 100%陽性であった．g 当りの菌数は 23～54,000 で他種動物材料の菌数に比べると一般に少なく，100以下 8 例，110～1,000は13例，1,100～1万6例，1.1万以上3例の成績であって，これらは前項の 5 例のウシの成績とほぼ似ている．牛舎別にみると，A牛舎10例の菌数は 1 例を除き大差のない菌数を示した．C及びE牛舎の例もほぼ同様の傾向を示している．しかしB及びD牛舎の例では菌数の分布の巾が A, C, E に比べて大である．

3. 再び人の糞便について

本材料についても大腸菌群の検査は省略し，腸球菌群のみの検査を行った．腸球菌群は100%陽性であり，出現菌数の順に50例の成績を示すと Table 24 の如くである．

Table 24. Number of enterococcus group bacteria per g in human feces.

| Sample No. | M.P.N. of enterococcus group bacteria | Sample No. | M.P.N. of enterococcus group bacteria | Sample No. | M.P.N. of enterococcus group bacteria |
|---|---|---|---|---|---|
| 1 | 49 | 18 | 70,000 | 35 | 2,400,000 |
| 2 | <230 | 19 | 79,000 | 36 | 2,400,000 |
| 3 | 230 | 20 | 79,000 | 37 | 2,400,000 |
| 4 | 2,300 | 21 | 130,000 | 38 | 2,400,000 |
| 5 | 3,300 | 22 | 130,000 | 39 | 2,400,000 |
| 6 | 3,300 | 23 | 240,000 | 40 | 3,300,000 |
| 7 | 4,900 | 24 | 240,000 | 41 | 3,500,000 |
| 8 | 11,000 | 25 | 240,000 | 42 | 3,500,000 |
| 9 | 11,000 | 26 | 330,000 | 43 | 5,400,000 |
| 10 | <23,000 | 27 | 330,000 | 44 | 7,900,000 |
| 11 | 24,000 | 28 | 330,000 | 45 | 24,000,000 |
| 12 | 33,000 | 29 | 330,000 | 46 | 24,000,000 |
| 13 | 33,000 | 30 | 330,000 | 47 | 24,000,000 |
| 14 | 33,000 | 31 | 330,000 | 48 | 24,000,000 |
| 15 | 33,000 | 32 | 790,000 | 49 | 54,000,000 |
| 16 | 49,000 | 33 | 1,300,000 | 50 | 54,000,000 |
| 17 | 70,000 | 34 | 2,200,000 | | |

菌数は49～54,000,000に分布し，一般にウシの例より多く分布の巾も大であった．しかし第1項の初生児及び成人の成績と比較すると，分布の巾はむしろ本例の方が小さい成績を示した．

III 小　　括

人及び各種動物の糞便55例では腸球菌群並びに大腸菌群とも全例陽性であった．これら両菌群の出現菌数の多少を比較すると，大腸菌群数が腸球菌群数より多い例数とその逆の例数との比は凡そ3：2の割となり，大腸菌群数の多い方が多数を占めた．また各5例の平均値においても大腸菌群数の多いのは，ウシ，ウマ，ヒツジ，ブタ，イヌ及び成人であったの対し，腸球菌群数の多いのはニワトリ，ネズミ，初生児と例数が少ない．なおこれらのうちイヌにおいて腸球菌群数は$10^7$～$10^8$，大腸菌群数は$10^6$～$10^8$で両菌群数とも最高値を示している．また腸球菌群数の最低を示したのはウシであった．

ウシ糞便30例の腸球菌群の検査結果も全例陽性であり，出現菌数は同一牛舎由来の材料では同程度に出現する傾向がみられた．

人糞便50例の成績も100%腸球菌群が陽性であり，出現菌数はウシに比べてはるかに多く，ほとんどが$10^3$～$10^7$の範囲にあった．

結局動物の個体別，種類別からみても，食品の第1次汚染源たる糞便中において，大腸菌群数は腸球菌群数より多く検出される例が多数で，総体的にみても大腸菌群数の方の多いことが認められ，このことは他の報告者の成績[23)27)61)64)66)]とも一致した．更に人及びウシの検査例数を追加して，特に腸球菌群のみを取上げて検討した結果，100%に検出されたのは勿論であるが，その出現菌数はウシではg当り23～54,000であったのに対し，人では49～54,000,000であり，人の糞便中の腸球菌群数は牛糞便のそれよりはるかに大であることを知った．

(V)　畜産食品及び糞便由来腸球菌群の同定分類並びに型別と由来材料との関係

(III)章及び(IV)章において述べた各種検査材料からそれぞれ分離したレンサ球菌について諸種の生物学的性状検査を行った．緒言において述べたように，わが国の報告にみられる腸球菌群の同定及び分類はBergey's Manualに準拠したものがほとんどであるが，著者はBergey's Manualのみによらず，近年提唱され始めた新しい分類の型式をも加味して分離腸球菌群の同定分類を試みた．また更にこのようにして得られた結果に基づいて，検査材料とそれらから出現した腸球菌群の型別との関連性を検討した結果，人糞便由来と動物糞便由来とでは，また非加熱食品由来と加熱食品由来とでは，各出現して来る腸球菌の型が異なること，すなわち検査材料により出現して来る腸球菌群の型に一定の傾向が認められたので，以下その成績結果について述べる．

I　実験材料及び方法

実験供試株は(III)章及び(IV)章で述べた畜産食品及び糞便から分離したものである．すなわち検査材料を推定並びに確認培地に培養して腸球菌群の陽性を確めた後，改めてこの推定培地から血液寒天平板培地に塗抹分離培養を行い，発育した集落を観察して異ると思われた集落からそれぞれ釣菌して菌株を得た．著者ら[21)]が先に報告した如く推定培地には，腸球菌群以外のレンサ球菌の発育もみられるので，血液寒天から釣菌した株の中にはこれらのレンサ球菌も含まれて来る．そこでこれらの分離株を再び腸球菌群の推定培地と確認培地に培養して十分に発育することを確めた後，それらの株を保存して以後の実験に供した．なお発育の疑わしい株も対照試験のため保存したので，結局合計968株のレンサ球菌が実験に供された．

生物学的性状の検査に当っては既報[21]の如く，カタラーゼ試験，グラム染色，溶血性，リトマス牛乳における発育態度，0.1%メチレン青牛乳，40%ウシ胆汁寒天，6.5%食塩ブイヨン及びpH 9.6のブイヨンにおける発育試験，10°C及び45°Cにおける発育，60°C 30分における耐熱性，ゼラチン液化能，各種糖類分解能及びLancefieldによるD群の沈降反応試験を行い，更にSKADHAUGE[80]によるPotassium tellurite(0.04%)に対する抵抗性，BARNES[4]によるTTC(0.01%)の還元性試験を行った，ついでこのようにして得られた試験成績から株の同定分類を行い，検査材料とそれらから出現して来た腸球菌群の型との関係を検討考察した．

## II 分離レンサ球菌の生物学的性状並びにその同定と分類

供試菌968株のカタラーゼ試験では全株が陰性を示した．グラム染色ではすべてが陽性の球菌で双球状あるいは連鎖状を示した．ウマ及びヒツジ血液寒天平板37°C 48時間培養における溶血態度は，4株がβ型を示したのみで残りの964株はα及びγ型を示した．β型の4株のうち，3株はウマ血液寒天平板上においてのみ（小林III型），1株はウマ及びヒツジの両血液寒天平板上でともに溶血を示した（小林II型）．しかも後者の株は培養24時間目においてαとβに解離する株であった．すなわち血液寒天平板上で継代培養を行い，発育したα，βの集落からαあるいはβの集落のみをそれぞれ釣菌して継代培養を繰り返し，100代迄分離培養を行ったが，依然としてβ集落からαとβ，α集落からもαとβが解離して出現した．このような現象は今泉ら[30]が報告した非定型的腸球菌にみられた態度と似ている．

一方岡本(1954)[63]は核酸を血液寒天培地に加えることにより，溶連菌のStreptolysin-Sの産出を増加せしめることから，溶連菌と非溶連菌との鑑別が可能であると報告している．よって岡本の実験方法に従いリボ核酸（キリンビールKK製）を加えた血液寒天培地及び核酸無添加の血液寒天培地を用意して，乳製品（1957年度分），粉乳（1958年度分）及び冬季生肉材料由来のレンサ球菌合計336株，更に対照として北大獣医学部家畜衛生学教室清水助教授より分与された溶血性レンサ球菌14株（C群4株，D群1株，E群2株，F群2株，G群1株，その他4株），東大農学部家畜細菌学教室尾形助教授より分与された溶血性腸球菌3株を加えて，これらにつきα，β溶血性の関係を検討した．その結果対照のβ溶血株では核酸加血液寒天培地上で，核酸無添加の培地におけるよりも溶血環が一層大きくなることが観察されたが，試験株のα型あるいはγ型のものがβ型と判定されるような成績は得られなかった．すなわち腸球菌群のα型ではたとえ培地に核酸を添加してもβ型と判定されるに至るような現象は認められないものと思われる．

リトマス牛乳においては酸産生，還元及び凝固を示したものが823株，凝固性を欠くもの103株，酸産生あるいは還元のみを示したものは42株であった．ゼラチン液化は100株にみられたが，これらの株はすべてリトマス牛乳のカゼインを完全に融解した．

0.1%メチレン青牛乳，40%ウシ胆汁血液寒天，6.5%食塩ブイヨン，pH 9.6のブイヨン，10°C及び45°C培養における発育態度，また60°C 30分における耐熱性等の成績はTable 25に示すとおりである．以上7種の抵抗性試験のうち，60°C 30分の耐熱性試験及び10°Cにおける発育試験を除いた5種の試験において，一致して陽性を示したものは873株であり，以下これらはすべて腸球菌群として取扱うことにする．他の95株は2種以上の試験において陰性を示したものであり，腸球菌群以外のレンサ球菌と考えられる．腸球菌群と判定した873株中18株は60°C 30分の熱抵抗性試験を欠いていたが，沈降反応その他の諸性状は他の腸球菌群と全く一致した．なお各材料から検出分離された株の腸球菌群あるいはその他のレンサ

Table 25. Biological characteristics of streptococcal strains isolated from milk, meat and their products and feces.

| Division | | | Udder milk | | Raw milk | | Dairy products (1957) | | Powdered milk (1958) | | Raw meat (Winter) | | Raw meat (Summer) | | Meat products | | Human and animal feces | | Bovine feces (1960) | | Human feces (1960) | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | E | S | E | S | E | S | E | S | E | S | E | S | E | S | E | S | E | S | E | S | E | S |
| | | | 6 | 5 | 98 | 4 | 52 | 15 | 155 | 5 | 53 | 56 | 113 | 1 | 28 | 1 | 76 | 2 | 124 | 4 | 168 | 2 | 873 | 95 |
| B.T.B. Azide Dextrose Broth | Yellow | + | 6 | 4 | 98 | 4 | 52 | 11 | 155 | 5 | 53 | 54 | 109 | 1 | 28 | • | 75 | 2 | 123 | • | 168 | 1 | 867 | 82 |
| | | ± | • | 1 | • | • | • | 4 | • | • | • | 2 | 4 | • | • | 1 | 1 | • | 1 | 4 | • | 1 | 6 | 13 |
| | Turbidity | + | 6 | 4 | 94 | 4 | 51 | 1 | 154 | 3 | 51 | 37 | 107 | 1 | 28 | • | 74 | 2 | 124 | 4 | 168 | 2 | 857 | 58 |
| | | ± | • | 1 | 4 | • | 1 | 4 | 1 | 2 | 2 | 18 | 6 | • | • | 1 | 2 | • | • | • | • | • | 16 | 26 |
| | | − | • | • | • | • | • | 10 | • | • | • | 1 | • | • | • | • | • | • | • | • | • | • | • | 11 |
| | Sediment | + | 6 | 5 | 98 | 4 | 52 | 15 | 155 | 5 | 53 | 56 | 113 | 1 | 28 | 1 | 76 | 2 | 124 | 4 | 168 | 2 | 873 | 95 |
| | | − | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| B.T.B. Azide Dextrose Agar | Yellow, small colony | | 6 | 4 | 92 | 3 | 52 | 6 | 146 | • | 51 | 24 | 109 | • | 28 | 1 | 69 | • | 113 | • | 167 | 1 | 833 | 39 |
| | Light yellow, pinpoint colony | | • | 1 | 6 | 1 | • | 9 | 9 | 5 | 2 | 32 | 4 | 1 | • | • | 7 | 2 | 11 | 4 | 1 | 1 | 40 | 56 |
| Growth at pH 9.6 | | + | 6 | • | 98 | • | 52 | 10 | 155 | • | 53 | 41 | 113 | 1 | 28 | • | 76 | • | 124 | • | 168 | • | 873 | 52 |
| | | − | • | 5 | • | 4 | • | 5 | • | 5 | • | 15 | • | • | • | 1 | • | 2 | • | 4 | • | 2 | • | 43 |
| Growth in 0.1% methylen blue milk | | + | 6 | 2 | 98 | 1 | 52 | 1 | 155 | • | 53 | 10 | 113 | • | 28 | • | 76 | • | 124 | • | 168 | 1 | 873 | 15 |
| | | − | • | 3 | • | 3 | • | 14 | • | 5 | • | 46 | • | 1 | • | 1 | • | 2 | • | 4 | • | 1 | • | 80 |
| Growth on 40% bile blood agar | | + | 6 | 3 | 98 | 2 | 52 | 15 | 155 | 5 | 53 | 56 | 113 | 1 | 28 | 1 | 76 | 2 | 124 | 4 | 168 | 2 | 873 | 91 |
| | | − | • | 2 | • | 2 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | 4 |
| Growth in 6.5% NaCl | | + | 6 | 2 | 98 | 1 | 52 | 9 | 155 | • | 53 | 26 | 113 | 1 | 28 | 1 | 76 | • | 124 | • | 168 | 1 | 873 | 41 |
| | | − | • | 3 | • | 3 | • | 6 | • | 5 | • | 30 | • | • | • | • | • | 2 | • | 4 | • | 1 | • | 54 |
| Survival at 60°C for 30 min. | | + | 6 | 2 | 98 | 1 | 52 | 11 | 155 | 5 | 53 | 16 | 110 | • | 28 | 1 | 74 | 1 | 114 | • | 165 | 1 | 855 | 38 |
| | | − | • | 3 | • | 3 | • | 4 | • | • | • | 40 | 3 | 1 | • | • | 2 | 1 | 10 | 4 | 3 | 1 | 18 | 57 |
| Growth at 45°C | | + | 6 | 3 | 98 | 4 | 52 | 3 | 155 | 5 | 53 | 9 | 113 | • | 28 | 1 | 76 | 1 | 124 | 4 | 168 | 2 | 873 | 32 |
| | | − | • | 2 | • | • | • | 12 | • | • | • | 47 | • | 1 | • | • | • | 1 | • | • | • | • | • | 63 |
| Growth at 10°C | | + | 6 | 3 | 98 | • | * | * | 155 | • | 53 | * | 113 | 1 | 28 | 1 | 76 | • | 124 | • | 168 | 2 | 821 | 7 |
| | | − | • | 2 | • | 4 | | | • | 5 | • | | • | • | • | • | • | 2 | • | 4 | • | • | • | 17 |
| Liquefaction of gelatin | | + | 1 | • | 14 | • | 3 | • | 2 | • | 13 | • | 33 | • | 4 | • | 10 | • | • | • | 20 | • | 100 | • |
| | | − | 5 | 5 | 84 | 4 | 49 | 15 | 153 | 5 | 40 | 56 | 80 | 1 | 24 | 1 | 66 | 2 | 124 | 4 | 148 | 2 | 773 | 95 |
| Hemolysis (horse and sheep blood agar) | | β | • | • | • | • | • | • | • | • | 1 | • | • | • | • | • | 1 | • | • | • | 2 | • | 4 | • |
| | | α～γ | 6 | 5 | 98 | 4 | 52 | 15 | 155 | 5 | 52 | 56 | 113 | 1 | 28 | 1 | 75 | 2 | 124 | 4 | 166 | 2 | 869 | 95 |

Notes. E : Enterococcus group. S : Streptococci other than enterococcus group. * : Not examined.

球菌としての区分はTable 25に示されるが，冬季生肉材料からの株には腸球菌群以外のレンサ球菌が特に多くみられるが，これは腸球菌群の検査にあたり，培地No. 1を用いたため他のレンサ球菌の発育を許した結果と考えられる．これらレンサ球菌の各種試験に対する成績を由来材料別にみると，pH 9.6のブイヨンにおける発育は乳製品（1957年），生肉（冬季及び夏季）由来株の一部を除いてすべてが陰性であり，0.1%メチレン青牛乳発育試験では，乳房採取乳，持込乳，乳製品（1957年），生肉（冬季）及び人糞便由来株の一部を除きすべてが陰性であった．それに反して40%ウシ胆汁血液寒天培地には乳房採取乳及び持込乳の一部を除きすべてが腸球菌群同様発育した．6.5%食塩ブイヨンにおける発育性及び60°C 30分の耐熱性試験では区々の成績を示した．45°Cにおける発育試験では乳製品（1957年）及び冬季生肉由来株に陰性例が多かった．要するに40%ウシ胆汁血液寒天培地ではほとんどの株が発育するため，腸球菌群との間に差が余り認められないが，0.1%メチレン青牛乳，次いで45°Cにおける発育には差が比較的認められた．これは既報[21]の成績とほぼ同じような結果である．

ゼラチン液化性の100株はウシ糞便以外の材料からすべて出現した．β型の腸球菌は生肉（冬季）から1株，人及び各種動物の糞便（ヒツジ）から1株，人糞便から2株の計4株である．なお腸球菌群とその他のレンサ球菌の腸球菌群推定培地及び確認培地における発育態度は表の上欄に示されるように区分された．

腸球菌群の型別については，糖及びアルコール類の分解態度からも試みられている[14)24)]．

Table 26. Fermentations of streptococci isolated.

| Division | | Udder milk | | Raw milk | | Dairy products (1957) | | Powdered milk (1958) | | Raw meat (Winter) | | Raw meat (Summer) | | Human and animal feces | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | E | S | E | S | E | S | E | S | E | S | E | S | E | S | E | S |
| | | 6 | 5 | 98 | 4 | 52 | 15 | 155 | 5 | 53 | 56 | 113 | 1 | 76 | 2 | 553 | 88 |
| Glucose | + | 6 | 5 | 98 | 4 | 52 | 9 | 155 | 5 | 53 | 50 | 113 | 1 | 76 | 2 | 553 | 76 |
| | − | • | • | • | • | • | 6 | • | • | • | 6 | • | • | • | • | • | 12 |
| Maltose | + | 6 | 5 | 98 | 4 | 52 | 13 | 155 | 5 | 53 | 47 | 113 | 1 | 76 | 1 | 553 | 76 |
| | − | • | • | • | • | • | 2 | • | • | • | 9 | • | • | • | 1 | • | 12 |
| Lactose | + | 6 | 2 | 96 | 3 | 52 | 7 | 152 | 5 | 50 | 22 | 101 | 1 | 76 | 1 | 533 | 41 |
| | − | • | 3 | 2 | 1 | • | 8 | 3 | • | 3 | 34 | 12 | • | • | 1 | 20 | 47 |
| Salicin | + | 6 | 4 | 97 | 4 | 52 | 11 | 154 | 2 | 53 | 37 | 111 | 1 | 76 | 1 | 549 | 60 |
| | − | • | 1 | 1 | • | • | 4 | 1 | 3 | • | 19 | 2 | • | • | 1 | 4 | 28 |
| Rhamnose | + | • | • | 16 | • | • | 2 | • | • | 1 | 2 | 14 | • | 6 | • | 37 | 4 |
| | − | 6 | 5 | 82 | 4 | 52 | 13 | 155 | 5 | 52 | 54 | 99 | 1 | 70 | 2 | 516 | 84 |
| Sucrose | + | 6 | 3 | 76 | 4 | 32 | 7 | 130 | 5 | 49 | 27 | 88 | 1 | 64 | 1 | 445 | 48 |
| | − | • | 2 | 22 | • | 20 | 8 | 25 | • | 4 | 29 | 25 | • | 12 | 1 | 108 | 40 |
| Glycerol | + | 4 | • | 85 | • | 34 | 5 | 114 | • | 39 | 1 | 81 | • | 52 | • | 409 | 6 |
| | − | 2 | 5 | 13 | 4 | 18 | 10 | 41 | 5 | 14 | 55 | 32 | 1 | 24 | 2 | 144 | 82 |
| Mannitol | + | 5 | 2 | 78 | • | 27 | 3 | 138 | • | 44 | 23 | 80 | 1 | 55 | • | 427 | 29 |
| | − | 1 | 3 | 20 | 4 | 25 | 12 | 17 | 5 | 9 | 33 | 33 | • | 21 | 2 | 126 | 59 |
| Sorbitol | + | 4 | 2 | 54 | • | 10 | • | 8 | • | 27 | 17 | 56 | • | 21 | • | 180 | 19 |
| | − | 2 | 3 | 44 | 4 | 42 | 15 | 147 | 5 | 26 | 39 | 57 | 1 | 55 | 2 | 373 | 69 |
| Arabinose | + | 2 | 2 | 16 | • | 34 | • | 112 | • | 11 | 5 | 12 | • | 43 | • | 230 | 7 |
| | − | 4 | 3 | 82 | 4 | 18 | 15 | 43 | 5 | 42 | 51 | 101 | 1 | 33 | 2 | 323 | 81 |
| Raffinose | + | 2 | 2 | 18 | 3 | 5 | 4 | • | 5 | 6 | 14 | 17 | 1 | 10 | 1 | 58 | 30 |
| | − | 4 | 3 | 80 | 1 | 47 | 11 | 155 | • | 47 | 42 | 96 | • | 66 | 1 | 495 | 58 |

Notes. E : Enterococcus group.
S : Streptococci other than enterococcus group.

著者も同じ目的をもって各種糖類及びアルコール類に対する試験を行った．1960年度検査分を除く腸球菌群及びその他のレンサ球菌の材料別の成績は Table 26 に示すとおりである．腸球菌群についてみると，すべてが Glucose（鹿印特級）及び Maltose（鹿印特級）を分解し，Salicin（鹿印特級）では99%以上，Lactose（鹿印特級）では96%以上を分解した．それに反して Rhamnose（武田最純）及び Raffinose（鹿印特級）では非分解株が多かった．その他の糖類では区々の成績を示した．これらの成績は前報[21]とほとんど同じ傾向にあった．

以上の如く Glucose, Maltose, Lactose, Salicin, Rhamnose 及び Raffinose に対してはほぼ一定した分解態度を示しているのでこれらの糖類を省略し，また Bergey's Manual によれば *Str. durans* の型別のため Mannitol 及び Sorbitol の，BARNES[4] その他[3)31)76)]によれば *Str. faecium* の型別のため Arabinose の分解の有無が鑑別点となっているので，1960年度に分離した肉製品，人及びウシ糞便由来レンサ球菌327株については特に Mannitol（鹿印特級），Sorbitol（井筒化学用），Arabinose（鹿印特級）の3種糖類を中心として，更に必要に応じては Sucrose (Merck) 及び Raffinose に対する分解能を検査した．その成績は Table 27 に示されるが，Table 26 における成績同様区々の成績を示した．なお腸球菌群の型別と糖分解能との関係については改めて後述する．

Table 27. Fermentations of streptococci isolated from meat products and feces (results in 1960).

| Division | | Meat products | | Bovine feces | | Human feces | |
|---|---|---|---|---|---|---|---|
| | | E | S | E | S | E | S |
| | | 28 | 1 | 124 | 4 | 168 | 2 |
| Mannitol | + | 24 | 1 | 102 | • | 138 | 2 |
| | − | 4 | • | 22 | 4 | 30 | • |
| Sorbitol | + | 12 | • | 61 | • | 50 | 1 |
| | − | 16 | 1 | 63 | 4 | 118 | 1 |
| Arabinose | + | 12 | • | 102 | 3 | 97 | 1 |
| | − | 16 | 1 | 22 | 1 | 71 | 1 |
| Raffinose | + | 2 | | 15 | | 11 | |
| | − | 1 | | 4 | | 9 | |
| Sucrose | + | 5 | | 1 | | 15 | |
| | − | 3 | | • | | • | |

Notes. E : Enterococcus group.
S : Streptococci other than enterococcus group.

Lancefield の沈降反応による分類試験は各材料由来株を糖分解能の成績を基にして型別し，それらの各型から数株づつを取上げ合計 228株について実施した．供試抗体血清は粉乳由来腸球菌株を使用して常法によりウサギを免疫して作製した．試験結果は Table 28 に示されるように，前記各種生物学的性状より腸球菌群と判定した207株はすべて D 群血清に反応し，その他のレンサ球菌と判定した21株中 8 株は反応陽性を，13株は陰性を示した．なおこの 8 株は種々の生物学的性状の結果から *Str. bovis* と同定された．

以上は Bergey's Manual の腸球菌群の同定分類形式によった試験成績であるが，近年この分類形式に批判的な報告がみられるに至った．すなわち SKADHAUGE[80], BARNES[4] その他の報告である．よって著者もこれらの報告を参考として，以上の腸球菌群の同定分類形式に Potassium tellurite に対する抵抗性試験並びに TTC に対する還元性試験をもつけ加えて腸

Table 28. Results of Lancefield's group D precipitation test.

| Division | No. of strains | Group D precipitation test | | | |
|---|---|---|---|---|---|
| | | Enterococcus group | | Streptococci other than enterococcus group | |
| | | + | − | + | − |
| Udder milk | 3 | 2 | 0 | 0 | 1 |
| Raw milk | 28 | 27 | 0 | 1 | 0 |
| Dairy products (1957) | 67 | 52 | 0 | 3 | 12 |
| Powdered milk (1958) | 38 | 35 | 0 | 3 | 0 |
| Raw meat (Winter) | 16 | 15 | 0 | 1 | 0 |
| 〃 〃 (Summer) | 26 | 26 | 0 | | |
| Human and animal feces | 50 | 50 | 0 | | |
| Total | 228 | 207 | 0 | 8* | 13 |

* : *Str. bovis.*

球菌群の同定型別を試みることとした．すなわち腸球菌群と判定した873株について Potassium tellurite（鹿印特級）に対する抵抗性，TTC (Merck) に対する還元性について試験した成績は Table 29 に示すとおり，0.04%の Potassium tellurite に耐えて発育陽性を示したものは合計 204株であったのに対し，発育陰性あるいは痕跡程度の発育に終ったものは各材料において何れも陽性株より多く，合計 669株であった．これらの差は粉乳，ウシ糞便において顕著であり，乳製品，人及び各種動物糞便，人糞便（1960 年度検査分）においても著しい．TTC (0.01%) の還元性も同じ結果を示した．

Table 29. Tolerance to potassium tellurite and reduction of TTC of enterococcus group isolated from milk, meat and their products and feces.

| Division | No. of strains | Tolerance to 0.04% potassium tellurite | | Reduction of TTC | |
|---|---|---|---|---|---|
| | | + | − | + | − |
| Udder milk | 6 | 3 | 3 | 3 | 3 |
| Raw milk | 98 | 52 | 46 | 52 | 46 |
| Dairy products (1957) | 52 | 9 | 43 | 9 | 43 |
| Powdered milk (1958) | 155 | 5 | 150 | 5 | 150 |
| Raw meat (Winter) | 53 | 20 | 33 | 20 | 33 |
| 〃 〃 (Summer) | 113 | 51 | 62 | 51 | 62 |
| Meat products | 28 | 12 | 16 | 12 | 16 |
| Human and animal feces | 76 | 14 | 62 | 14 | 62 |
| Bovine feces (1960) | 124 | 1 | 123 | 1 | 123 |
| Human feces (1960) | 168 | 37 | 131 | 37 | 131 |
| Total | 873 | 204 | 669 | 204 | 669 |

以上両者の試験において陽性を示した 204株は *Str. faecalis* 及びその varieties であり，ゼラチン液化の 100株は *Str. faecalis* var. *liquefaciens*, β溶血（小林 III型）の 3株は *Str. faecalis* var. *zymogenes*, 残りの101株は *Str. faecalis* とすることが妥当と考えられる．一

Table 30. Classification of streptococci isolated from milk, meat and their products and feces.

| Division | No. of strains | *Str. faecalis* | *Str. faecalis* var. *liquefaciens* | *Str. faecalis* var. *zymogenes* | *Str. faecium* | *Str. durans* | Unidentified enterococci | *Str. bovis* | Unidentified streptococci |
|---|---|---|---|---|---|---|---|---|---|
| Udder milk | 11 | 2 | 1 | | 2 | 1 | | 1 | 4 |
| Raw milk | 102 | 38 | 14 | | 16 | 17 | 13 | 3 | 1 |
| Butter (1957) | 41 | 2 | 2 | | 17 | 5 | | 3 | 12 |
| Powdered milk (1957) | 26 | 4 | 1 | | 17 | 3 | 1 | | |
| 〃 〃 (1958) | 160 | 3 | 2 | | 111 | 7 | 32 | 5 | |
| Raw meat (Winter) | 109 | 6 | 13 | 1 | 11 | 3 | 19 | 5 | 51 |
| 〃 〃 (Summer) | 114 | 18 | 33 | | 12 | 23 | 27 | | 1 |
| Meat products | 29 | 8 | 4 | | 12 | 1 | 3 | | 1 |
| Various animal feces | 63 | 3 | 4 | | 35 | 19 | 1 | | 1 |
| Human feces (1957–'58) | 15 | 1 | 6 | | 7 | | | | 1 |
| Human feces (1960) | 170 | 15 | 20 | 2 | 96 | 9 | 26 | | 2 |
| Bovine feces ( 〃 ) | 128 | 1 | | | 102 | 5 | 16 | 4 | |
| Total | 968 | 101 | 100 | 3 | 438 | 93 | 138 | 21 | 74 |

Table 31. Relationship between classification and fermentation of strains of enterococcus group isolated from milk, meat and their products and feces.

| Division | *Str. faecalis* | | | | *Str. faecalis* var. *liquefaciens* | | *Str. faecalis* var. *zymogenes* | *Str. durans* | | | | *Str. faecium* | | | | | | | Unidentified enterococci | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Lactose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | +/− | |
| Salicin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | +/− | |
| Sucrose | + | + | − | − | + | + | + | + | + | − | − | +/− | +/− | + | + | +/− | +/− | + | +/− | +/− | − | + | + | + | +/− | |
| Glycerol | + | + | + | + | + | + | + | + | − | − | + | + | − | + | − | − | + | + | + | − | + | + | + | − | +/− | |
| Mannitol | + | + | + | + | + | + | + | − | − | − | − | + | + | + | + | − | − | − | + | + | + | − | − | + | +/− | |
| Sorbitol | + | + | + | − | + | + | + | − | − | − | − | − | − | + | + | − | − | + | − | − | + | − | + | + | +/− | |
| Arabinose | − | + | − | − | − | + | − | − | − | − | − | + | + | + | + | + | + | + | − | − | − | − | − | − | − | |
| Raffinose | − | − | − | − | − | − | − | − | − | − | − | +/− | +/− | +/− | − | +/− | +/− | − | +/− | +/− | − | + | − | − | +/− | |
| Udder milk | 2 | | | | 1 | | | | 1 | | | | 1 | 1 | | | | | | | | | | | | 6 |
| Raw milk | 31 | | 5 | 2 | 14 | | | | 5 | 2 | 10 | 12 | | 2 | | | 2 | | 6 | 3 | | 1 | | | 3 | 98 |
| Dairy products | 2 | | 4 | | 3 | | | 2 | | 1 | 5 | 10 | 6 | | | 10 | 7 | 1 | 1 | | | | | | | 52 |
| Powdered milk | 3 | | | | 1 | 1 | | 1 | | | 6 | 71 | 30 | | 3 | 7 | | | 28 | | | | | | 4 | 155 |
| Raw meat(Winter) | 6 | | | | 13 | | 1* | | 2 | | 1 | 3 | 4 | 1 | | | 3 | | 9 | 2 | 1 | 1 | | 3 | 3 | 53 |
| 〃 〃 (Summer) | 16 | | 2 | | 33 | | | 1 | 10 | 9 | 3 | 8 | | 2 | | | 2 | | 11 | 2 | 1 | 1 | | | 12 | 113 |
| Human and animal feces | 3 | 1 | | | 9 | 1 | | 1* | 12 | 3 | 3 | 26 | 9 | 6 | | | 1 | | | | | | 1 | | | 76 |
| Meat products | 5 | | 3 | | 4 | | | | 1 | | | 11 | | | | | 1 | | 1 | | | 2 | | | | 28 |
| Bovine feces | 1 | | | | | | | | 5 | | | 40 | | 60 | | | 2 | | 1 | | | 15 | | | | 124 |
| Human feces | 14 | 1 | | | 20 | | 2* | | 9 | | | 73 | | 13 | | | 10 | | 15 | | | 11 | | | | 168 |
| Total | 101 | | | | 100 | | 3 | 93 | | | | 438 | | | | | | | 138 | | | | | | | 873 |

* β hemolysis.

方両試験において陰性を示した669株には *Str. durans* 及び *Str. faecium* が含まれる. BARNES[4], その他[3)31)76)]によれば *Str. faecium* は Potassium tellurite 及び TTC の両試験で陰性を示すと同時に Arabinose を分解することが重要な鑑別点として挙げられている. よって Potassium tellurite 及び TTC の両試験で陰性を示した669株中 Arabinose 分解株を *Str. faecium* とした. *Str. durans* は Bergey's Manual によれば β溶血を有し, Mannitol 及び Sorbitol 非分解が鑑別点とされているが, 更に Raffinose も一般に非分解とされている. β溶血に関しては異論が多く, SHATTOCK,[76] SEELEMANN[74] によれば溶血性を欠くものもあるといわれる. これらの報告に従い Potassium tellurite 及び TTC の両試験で陰性を示し, Mannitol, Sorbitol, Arabinose 及び Raffinose 非分解株を *Str. durans* とした. この中にはαとβに解離した1株 (小林 II型) も含まれる. また糖類分解能の成績から, *Str. faecium* にも *Str. durans* にも属しない残りの株は Unidentified enterococci とした.

以上の型別によって可検菌968株を分類した結果は Table 30 に示されるように, 腸球菌群は *Str. faecalis* 101株, *Str. faecalis* var. *liquefaciens* 100株, *Str. faecalis* var. *zymogenes* 3株, *Str. durans* 93株, *Str. faecium* 438株, Unidentified enterococci 138株の計873株, 腸球菌群以外のレンサ球菌は *Str. bovis* 21株, 同定不能の不明レンサ球菌74株の計95株となり, 腸球菌群では *Str. faecium* が圧倒的に多く, ついで Unidentified enterococci であり, *Str. faecalis*, *Str. faecalis* var. *liquefaciens* 及び *Str. durans* はほぼ同じく, β溶血性を示す *Str. faecalis* var. *zymogenes* は極めて少なかった.

なお型別した腸球菌群の糖類分解能の成績を改めて検討すると, Table 31 に示されるように供試873株はすべて Glucose, Maltose を分解し, 大部分の株は Lactose, Salicin を分解した. Potassium tellurite 及び TTC の両試験で陽性を示す *Str. faecalis* 及びその varieties の *liquefaciens* と *zymogenes* の3型のいづれかに属する204株はすべて Glycerol, Mannitol を分解し, Raffinose は非分解であった. なお Sorbitol に対しては2株のみ陰性, Sucrose に対しては16株が陰性であり, Arabinose に対しては4株が陽性を示した. 以上のように *Str. faecalis* 及びその varieties の糖分解能の態度はほぼ一定しているのに比べて, *Str. durans* の93株では, Mannitol, Sorbitol, Arabinose 及び Raffinose に対して陰性であった他は Sucrose, Glycerol では区々の成績を示した. また *Str. faecium* の438株では Glucose, Maltose, Lactose, Salicin の他 Arabinose の5種糖類に対しすべて陽性を示したが, その他の糖類に対しては一定の傾向が見出されず区々の成績を示した.

III 各種検査材料における腸球菌群の型別による検出状況

1. 畜産食品において

食品類は種類, 成分が多種多様の上更に季節, 保存方法等環境による影響も受け易い. また畜産食品は生鮮品と加熱加工品とに大別されるから, このような性状の差により検出される腸球菌群の型に差が認められるかどうかを, 前項の同定分類成績に基づいて検討した.

TTC 及び Potassium tellurite の両試験に陽性を示した *Str. faecalis*, *Str. faecalis* var. *liquefaciens* 及び *Str. faecalis* var. *zymogenes* の3種は faecalis 系として, また *Str. durans* 及び Unidentified enterococci はその他として一括し, 結局 faecalis 系, *Str. faecium*, その他の3群に分け, もってこれら群の単独, 2群混合及び3群混合の際の出現状況を材料別に検討した. その成績は Table 32 に示される. なお faecalis 系とした *Str. faecalis* 及び *Str. faecalis* var. *liquefaciens* それぞれ単独の出現状況を参考のため特に下欄に示した.

Table 32. Distribution of species of enterococcus group isolated in milk, meat and their products.

| Division | Udder milk | Raw milk | Raw meat (Winter) | Raw meat (Summer) | Butter (1957) | Powdered milk (1957) | Powdered milk (1958) | Meat products |
|---|---|---|---|---|---|---|---|---|
| No. of samples inspected | 200 | 50 | 60 | 40 | 44 | 37 | 81 | 39 |
| No. of enterococcus group positive samples | 6 | 50 | 37 | 40 | 19 | 22 | 81 | 9 |
| Faecalis group | 3 | 20 | 9 | 9 | 2 | 4 | • | 2 |
| 〃 〃 + Others | • | 12 | 8 | 17 | • | • | • | 1 |
| 〃 〃 + *Str. faecium* | • | 5 (76%) 38 | 3 (54%) 20 | 3 (87.5%) 35 | 2 | 1 | 4 | 2 |
| 〃 〃 + 〃 〃 + Others | • | 1 | • | 6 | • | • | 1 | 1 |
| *Str. faecium* | 2 | 4 | 5 | 1 | 10 (73.6%) 14 | 13 (68.1%) 15 | 47 (88.8%) 72 | 2 |
| 〃 〃 + Others | • | 1 | 1 | • | 2 | 1 | 20 | • |
| Others | 1 | 7 | 11 | 4 | 3 | 3 | 9 | 1 |
| *Str. faecalis* | 2 | 14 | 4 | 3 | 1 | 4 | • | 2 |
| *Str. faecalis* var. *liquefaciens* | 1 | 6 | 5 | 5 | 1 | • | • | • |

Notes. Faecalis group : *Str. faecalis*, *Str. faecalis* var. *liquefaciens* and *Str. faecalis* var. *zymogenes*.
Others : *Str. durans* and unidentified enterococci.

*Str. faecalis* var. *zymogenes* は冬季生肉材料1例からのみ，他型と混合の状態で検出されたのにすぎなかったので省略した．

生材料である乳房直接採取乳は陽性例数も少ないため比較出来ないが，プラント持込乳において faecalis 系と *Str. faecium* の検出状況を比較すると，faecalis 系は単独，混合をあわせて検査材料の 76% (38/50) から検出されているのに対し，*Str. faecium* は同じく合計22% (11/50) のみであり，前者の検出率が大である．生肉（冬季及び夏季）においても faecalis 系の検出率はそれぞれ 54% (20/37)，87.5% (35/40) であり，*Str. faecium* の 24.3% (9/37)，25% (10/40) に比べて高い成績を示した．

一方製造工程中に加熱されるバター及び粉乳（1957年度検査分）においては逆に *Str. faecium* が多く，それぞれ 73.6% (14/19)，68.1% (15/22) であり，faecalis 系の 21% (4/19)，22.7% (5/22) に比べて高い成績であった．また1958年度検査分の粉乳も同様 *Str. faecium* が 88.8% (72/81) を示し，faecalis 系の検出率に比して圧倒的に高かった．肉製品では各々の陽性例数が少ないため，乳製品におけるような比較は出来なかった．

2. 糞便において

人及び各種動物の糞便材料についても前項同様の検討を行った．各群の検出状況は Table 33 に示すとおりである．

Table 33. Distribution of species of enterococcus group isolated in human and various animal feces.

| Division | Various animal feces | Human feces 1957～'58 | Human feces 1960 | Bovine feces |
|---|---|---|---|---|
| No. of samples inspected | 45 | 10 | 50 | 30 |
| No. of enterococcus group positive samples | 45 | 10 | 50 | 30 |
| Faecalis group | 4 | 6 | 1 | • |
| 〃 〃 + Others | 1 | • 7 (70%) | 1 30 (60%) | • |
| 〃 〃 + *Str. faecium* | 2 | 1 | 12 | 1 |
| 〃 〃 + 〃 〃 + Others | • | • | 16 | • |
| *Str. faecium* | 24 27 (60%) | 3 4 (40%) | 15 47 (94%) | 16 29 (96.6%) |
| 〃 + Others | 1 | • | 4 | 12 |
| Others | 13 | • | 1 | 1 |
| *Str. faecalis* | 2 | • | • | • |
| *Str. faecalis* var. *liquefaciens* | 2 | 6 | • | • |

Notes. Faecalis group : *Str. faecalis*, *Str. faecalis* var. *liquefaciens* and *Str. faecalis* var. *zymogenes*.
Others : *Str. durans* and unidentified enterococci.

各種動物の糞便材料においては faecalis 系よりも *Str. faecium* の検出率が高く，60% (27/45) に出現した．1957年度検査分の人糞便材料では例数は少ないが，faecalis 系が多かった．1960年度検査分の人糞便材料では，faecalis 系の 60% (30/50) に対し *Str. faecium* は更に多く 94% (47/50) を示した．ウシ糞便材料では faecalis 系の出現がわずか1例のみに対し，

*Str. faecium* は 96.6%（29/30）の割に検出された．

以上の成績を要約すると，生食品類からは faecalis 系の，加熱食品類では *Str. faecium* の検出率が高い．糞便においては各種動物，特にウシでは *Str. faecium* の検出率が高いのに対し，人では faecalis 系と *Str. faecium* が同程度に検出される傾向にある．

## IV　粉乳由来腸球菌群の型と工場製品別による検出状況

1958年度検査分の粉乳は，北海道内5カ所の各乳製品工場より10月1日から月末迄の1月の期間中に，それぞれ8～20日間ほぼ連日採取送付されて来たものである（Table 11 参照）．よって連日どのような状態で腸球菌群が検出され，しかも各工場別により検出される腸球菌群の菌型に特徴があるか否かを検討した．連日の検出状況は Table 11 に示されたように，腸球菌群は何れの工場を問わず全製品から検出され，その菌数も大部分が500以下の成績である．

次に検査材料から分離されたレンサ球菌160株について同定型別を行った成績は Table 34 に示されるように，*Str. faecalis* 3株，*Str. faecalis* var. *liquefaciens* 2株，*Str. durans* 7株，*Str. faecium* 111株，Unidentified enterococci 32株，*Str. bovis* 5株であった．各工場製品別に菌型の検出状況をみると，*Str. faecalis* 及び *Str. faecalis* var. *liquefaciens* は B 及び C 工場製品のみに，*Str. durans* は E 工場製品のみに検出された．以上に対して *Str. faecium* は5工場の製品すべてにみられ，88.8%（72/81）の陽性率であり，B,C,D 工場ではそれぞれ100%を示した．Unidentified enterococci も全工場製品から検出されたが，特に A工場製品に多かった．*Str. bovis* は3工場の製品から検出されている．

なお各工場製品とも *Str. faecium* が多数検出されているが，この *Str. faecium* が更に各種糖類分解能により型別されることは既に述べた（Table 31 参照）．それで同じ *Str. faecium* が検出された各工場製品でも *Str. faecium* を更に型別したとき，工場別に差があるかどうかとの考えのもとに，糖分解能による型別と各工場製品との関係を調べた．その成績は Table 35 に示される．すなわち *Str. faecium* その他腸球菌群は14型，*Str. bovis* は2型に分けられたが，A 工場では第5，第7及び第12型の3型に，B 工場は第5型，C 工場は第6型，D 工場は第5及び第6型．E 工場は第3型というように．工場により特異的な型の株が検出されている．また出現株数の最も多かった型は，*Str. faecium* の第5型であり，特に A,B,D 工場製品に多かったが，5工場製品のすべてから検出されているので粉乳製品においては最も普遍的にみられる型であるかも知れない．

## V　小　　括

畜産食品及び糞便材料から分離したレンサ球菌968株について同定分類した結果，腸球菌群は873株でその内訳は，*Str. faecalis* 101株，*Str. faecalis* var. *liquefaciens* 100株，*Str. faecalis* var. *zymogenes* 3株，*Str. durans* 93株，*Str. faecium* 438株及び Unidentified enterococci 138株であった．腸球菌群以外のレンサ球菌は95株でその内訳は，*Str. bovis* 21株，不明レンサ球菌74株である．以上の分類成績をみると *Str. faecium* が最も多く検出されているが，本型菌について型別した報告は未だ我国においてはみられない．

次に腸球菌群を faecalis 系（*Str. faecalis* 及びその varieties），*Str. faecium* 及びその他（*Str. durans* と Unidentified enterococci）の3群に大別してこれらの群が検査材料から検出される状態を検討した．その結果畜産食品において，生乳，生肉では faecalis 系が *Str.*

Table 34. Distribution of species of streptococci in powdered milk differentiated with each dairy plant.

| Dairy plant | No. of | | *Str. faecalis* | | *Str. faecalis* var. *liquefaciens* | | *Str. durans* | | *Str. faecium* | | Unidentified enterococci | | *Str. bovis* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | samples inspected | strains isolated | Samples | Strains | Samples | Strains | Samples | Strains | Samples | Strains | Samples | Strains | Samples | Strains |
| A | 24 | 52 | • | • | • | • | • | • | 19 | 24 | 19 | 26 | 1 | 2 |
| B | 12 | 34 | 2 | 2 | 2 | 2 | • | • | 12 | 29 | 1 | 1 | • | • |
| C | 17 | 31 | 1 | 1 | • | • | • | • | 17 | 27 | 1 | 1 | 2 | 2 |
| D | 20 | 29 | • | • | • | • | • | • | 20 | 27 | 1 | 1 | 1 | 1 |
| E | 8 | 14 | • | • | • | • | 6 | 7 | 4 | 4 | 3 | 3 | • | • |
| Total | 81 | 160 | 3 | 3 | 2 | 2 | 6 | 7 | 72 | 111 | 25 | 32 | 4 | 5 |

Notes. Samples : No. of samples detected.
Strains : No. of strains isolated.

Table 35. Relationship between milk powder of different dairy plants and subtyping by fermentation of streptococci isolated from milk powder.

| Subtype No. / Division | *Str. faecalis*, *Str. faecalis* var. *liquefaciens* | | *Str. durans* | | *Str. faecium* | | | | | | | Unidentified enterococci | | | *Str. bovis* | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | |
| Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Lactose | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | |
| Salicin | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | |
| Rhamnose | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | |
| Sucrose | + | + | − | + | + | + | − | + | + | − | − | + | − | + | + | + | |
| Glycerol | + | + | + | + | + | − | + | − | − | − | − | + | + | − | − | − | |
| Mannitol | + | + | − | − | + | + | + | − | + | + | − | + | − | + | − | − | |
| Sorbitol | + | + | − | − | − | − | − | − | + | − | − | − | − | − | − | − | |
| Arabinose | − | + | − | − | + | + | + | + | + | + | + | − | − | − | − | − | |
| Raffinose | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | + | |
| Dairy plant A | • | • | • | • | 14 | • | 10 | • | • | • | • | 26 | • | • | 2 | • | 52 |
| 〃 B | 3 | 1 | • | • | 25 | • | • | 4 | • | • | • | 1 | • | • | • | • | 34 |
| 〃 C | 1 | • | • | • | 1 | 20 | • | • | 3 | 3 | • | • | • | 1 | 1 | 1 | 31 |
| 〃 D | • | • | • | • | 17 | 6 | 2 | 2 | • | • | • | 1 | • | • | • | 1 | 29 |
| 〃 E | • | • | 6 | 1 | 2 | 1 | • | • | • | • | 1 | • | 3 | • | • | • | 14 |
| Total | 4 | 1 | 6 | 1 | 59 | 27 | 12 | 6 | 3 | 3 | 1 | 28 | 3 | 1 | 3 | 2 | 160 |

*faecium* より多く，製造工程中に加熱される乳製品では逆に *Str. faecium* が faecalis 系より多い成績を得，特に粉乳（1958年度検査分）においてこのことが顕著であった．但し製造工程中においてやはり加熱される肉製品では乳製品におけるような成績が得られなかった．

糞便材料において人では faecalis 系と *Str. faecium* の両方が高率に検出された．しかるに動物では *Str. faecium* の検出率が高く，特に牛では97%の高率であった．以上から検査材料の種類により検出される腸球菌群の型に違いがあり，しかも一定した出現傾向があることを知った．

更に北海道内5カ所の乳製品工場から毎日連続的に得られた粉乳由来レンサ球菌160株について同定型別を行い，特にその検出状況を各工場別に観察した結果，*Str. faecium* が多く，しかも本型菌は検出率に差は認められたが，各工場製品から普遍的に検出された．また糖類分解能により，*Str. faecium* その他を16型に分け，前同様これらと各工場製品との関係を検討した処，例えば同じ *Str. faecium* の検出された工場製品でも，工場によりある特定の菌型が特異的にしかも毎日連続して出現していることを知った．

### (VI) 腸球菌群の熱抵抗性試験

前章において述べたように，全く加熱しない検査材料たる生乳，生肉では faecalis 系が，これに対して加熱材料たる乳製品では *Str. faecium* が優勢に出現する傾向が認められたことから，このような菌型による出現傾向の差は，それぞれの菌型株の耐熱性の差によるものではなかろうかと推定するに至った．そこで *Str. faecium* と faecalis 系の *Str. faecalis* 及び *Str. faecalis* var. *liquefaciens* の3菌型株を選び，種々の条件下における熱抵抗性試験を行った．

#### I 実験供試株及び浮遊液

実験供試株として加熱の影響を受けていない生乳（プラント持込乳）及び生肉（夏季）から分離した *Str. faecalis*（以下 *faecalis* という），*Str. faecalis* var. *liquefaciens*（以下 *liquefaciens* という）及び *Str. faecium*（以下 *faecium* という）をそれぞれ各5株合計30株を選出した．

耐熱性試験用の浮遊液基質として脱脂乳及び Sφrensen の燐酸緩衝液（pH7.0）の2種を用い，それぞれ10mlづつ中試験管に分注して滅菌したものを用意した．

#### II 実験方法及び判定

感作温度は63°C及び75°Cの2種とし，恒温水槽を使用して温度誤差は ±0.5°C 以内に調節した．

作用時間は63°Cで30分及び60分，75°Cでは15分及び30分である．すなわちこれらは牛乳の低温殺菌法と同様のまたはそれ以上の加熱試験である．

浮遊液内における菌数は ml 当り100万台及び1万台になるように調製した．すなわちブイヨン 37°C 20時間培養菌を 4°C の氷室に納めて一旦冷却した後取出し，所定の菌数となるよう緩衝液で適宜稀釈してから接種調製した．他方調製と同時に同じ稀釈菌液から平板培養法による生菌数の計算を行い確実を期した．

所定の菌数を接種した中試験管をそれぞれの温度に一定時間作用させた後直ちに流水で冷却して生残の判定に供した．各供試菌株の生残判定は，脱脂乳浮遊液では 37°C の孵卵器内に納置して増菌培養を行い，培地の凝固及び血液寒天平板培地に培養することにより，緩衝

液の方はブイヨンによる増菌培養及び血液寒天平板培地に培養することにより確認した．これら増菌及び血液寒天培地への培養は5日目まで反復試験を行って生残を観察した．

## III 実 験 成 績

熱抵抗性試験は温度，時間，浮遊液基質及び菌数4種の組合せにより，1供試株当り16通りの試験が行われた．生乳由来株についての実験成績はTable 36に示されるとおりである．浮遊液菌数は *liquefaciens* が100万台でml当り329～400万，1万台はその1/100の3.29～4.00万であった．*faecalis* は同じく176～370万及び1.76～3.70万，*faecium* は325～370万及び3.25～3.70万であり，全体として176～400万及び1.76～4.00万の間に分布していた．これらの菌数は検定の結果同一母集団に属することが認められた ($P<0.01$)．

熱抵抗性試験の結果，菌数100万台において63°C 30分加熱では全供試株が浮遊液基質たる脱脂乳及び緩衝液の如何を問わず生残した．また63°C 60分においても全供試株が生残した．しかし両種浮遊液すべてに生残出来たのは *faecium* のみで，*liquefaciens* 及び *faecalis* では何れか一方の浮遊液にのみ生残した例もみられた．75°C 15分では何れか一方の浮遊液でのみ，*liquefaciens* は2株，*faecalis* は3株より生残出来なかったのに対し，*faecium* は5株すべてが生残した．75°C 30分では何れの菌型においても生残した株は1例もみられなかった．

菌数1万台のものにおいて63°C 30分では *liquefaciens* は1株のみ生残したのに対し，*faecalis* 及び *faecium* は全株生残した．60分の加熱では *liquefaciens* 株の生残はなく，*faecalis* は1株のみであったのに対し，*faecium* は全株生残した．75°C 15分では *liquefaciens* 及び *faecium* がそれぞれ1株づつ生残した．しかし *liquefaciens* の生残例は以上の実験結果からみて実験誤差とも考えられる．75°C 30分では全株死滅していた．

熱抵抗性試験の結果は以上の如く，浮遊液菌数100万台では75°C 15分，1万台では63°C 30分及び60分の成績にみられるように，明らかに菌型による熱抵抗性に差が認められた．すなわち *liquefaciens* の熱抵抗性が最も弱く，*faecium* が最も強く，*faecalis* はその中間に位することを実験的にも立証することが出来た．

生肉由来株について行った熱抵抗性試験の成績はTable 37に示すとおりである．供試15株の浮遊液菌数は100万台では181～355万，1万台では1.81～3.55万であって前記生乳由来株についての実験例における菌数と大体一致した．試験結果は菌数100万台のものは63°C 30分の加熱では全株生残し，60分においても *liquefaciens* 及び *faecalis* が1株づつ死滅したのみであった．75°C 15分では *liquefaciens* 及び *faecalis* が2株づつ，*faecium* は4株が生残した．30分では *faecium* の2株のみが生残を示した．

菌数1万台において63°C 30分加熱では *liquefaciens* 及び *faecalis* が1株づつ，*faecium* は3株が生残したのみであった．75°C 15分及び30分では全株死滅した．

生肉由来株では以上の如く浮遊液菌数100万台においては *liquefaciens* と *faecalis* の間に差が認められなかった．しかしこの2者と *faecium* との間には75°C 15分及び30分加熱の成績にみられるように差が生じた．一方菌数1万台において63°C 30分加熱では *liquefaciens* と *faecalis* との間にかなり明らかな差が認められ，更に60分加熱の成績では *faecalis* と *faecium* との間に差を生じた．結局生肉由来株の熱抵抗性試験でも生乳由来株におけると同様3菌型の間に明らかに差が認められ，*liquefaciens* の熱抵抗性が最も弱く，*faecium* が最も強い結果を示した．

Table 36. Thermoresistance tests of *Str. faecalis*, *Str. faecalis* var. *liquefaciens* and *Str. faecium* isolated from raw milk.

| Type | Strain No. | Level of million (No. of bacteria/ml) | 63°C | | 75°C | | Level of 10 thousand (No. of bacteria/ml) | 63°C | | 75°C | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 30′ | 60′ | 15′ | 30′ | | 30′ | 60′ | 15′ | 30′ |
| *Str. faecalis* var. *liquefaciens* | 1 | 3. 29 million | ++ | ++ | +° | − | 32. 9 thousand | − | − | − | − |
| | 2 | 3. 34 | ++ | +° | − | − | 33. 4 | − | − | − | − |
| | 3 | 3. 37 | ++ | +° | + | − | 33. 7 | − | − | + | − |
| | 4 | 3. 71 | ++ | +° | − | − | 37. 1 | + | − | − | − |
| | 5 | 4. 00 | ++ | ++ | − | − | 40. 0 | − | − | − | − |
| *Str. faecalis* | 11 | 1. 76 | ++ | ++ | − | − | 17. 6 | + | − | − | − |
| | 12 | 2. 04 | ++ | ++ | + | − | 20. 4 | + | +° | − | − |
| | 13 | 2. 57 | ++ | + | +° | − | 25. 7 | + | − | − | − |
| | 14 | 2. 87 | ++ | +° | − | − | 28. 7 | + | − | − | − |
| | 15 | 3. 70 | ++ | ++ | +° | − | 37. 0 | + | − | − | − |
| *Str. faecium* | 21 | 3. 25 | ++ | ++ | +° | − | 32. 5 | ++ | + | − | − |
| | 22 | 3. 26 | ++ | ++ | + | − | 32. 6 | ++ | + | − | − |
| | 23 | 3. 34 | ++ | ++ | + | − | 33. 4 | ++ | + | − | − |
| | 24 | 3. 55 | ++ | ++ | + | − | 35. 5 | + | + | − | − |
| | 25 | 3. 70 | ++ | ++ | + | − | 37. 0 | + | + | + | − |

Notes. Used suspension : Sφrensen's phosphate buffer solution (pH 7. 0) and skim milk.
++ : Survive in both buffer solution and skim milk.
+ : Survive in skim milk only.
+° : Survive in buffer solution only.

Table 37. Thermoresistance tests of *Str. faecalis*, *Str. faecalis* var. *liquefaciens* and *Str. faecium* isolated from raw meat.

| Type | Strain No. | Level of million (No. of bacteria/ml) | 63°C | | 75°C | | Level of 10 thousand (No. of bacteria/ml) | 63°C | | 75°C | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 30′ | 60′ | 15′ | 30′ | | 30′ | 60′ | 15′ | 30′ |
| *Str. faecalis* var. *liquefaciens* | 6 | 1.81 million | ++ | + | − | − | 18.1 thousand | − | − | − | − |
| | 7 | 2.86 | ++ | + | − | − | 28.6 | − | − | − | − |
| | 8 | 3.22 | + | + | − | − | 32.2 | + | + | − | − |
| | 9 | 3.50 | ++ | ++ | + | − | 35.0 | − | − | − | − |
| | 10 | 3.55 | + | − | + | − | 35.5 | − | − | − | − |
| *Str. faecalis* | 16 | 2.23 | + | ++ | − | − | 22.3 | − | − | − | − |
| | 17 | 2.45 | ++ | + | − | − | 24.5 | ++ | − | − | − |
| | 18 | 3.00 | + | + | + | − | 30.0 | + | − | − | − |
| | 19 | 3.09 | ++ | − | + | − | 30.9 | + | − | − | − |
| | 20 | 3.43 | ++ | + | − | − | 34.3 | ++ | + | − | − |
| *Str. faecium* | 26 | 2.31 | + | + | + | − | 23.1 | + | − | − | − |
| | 27 | 2.81 | ++ | + | ++ | − | 28.1 | − | − | − | − |
| | 28 | 3.10 | ++ | ++ | + | + | 31.0 | + | + | − | − |
| | 29 | 3.15 | ++ | + | + | ++ | 31.5 | + | + | − | − |
| | 30 | 3.30 | ++ | ++ | − | − | 33.0 | ++ | + | − | − |

Note. Signs employed in this table are those in table 36.

IV 小 括

生乳及び生肉からそれぞれ分離した *Str. faecalis, Str. faecalis* var. *liquefaciens* 及び *Str. faecium* 各5株計30株を供試菌として感作温度，時間，浮遊液基質及び菌数を組合せて種々の条件下にてこれら菌株の熱抵抗性試験を行った．その結果生乳あるいは生肉由来を問わず *Str. faecalis* var. *liquefaciens* の熱抵抗性が最も弱く，*Str. faecium* が最も強く，*Str. faecalis* がこの中間に位する成績を得た．また基質中に含まれる菌数の多少により，加熱による菌の死滅態度は異なり，含有菌数の少ないもの程死滅し易い結果を得た．

## (VII) 保存による腸球菌群の生残試験

糞便及び食品中に含まれる腸球菌群並びに大腸菌群は時日の経過とともにその菌数はどのように変化し，またどちらが長く生残するかを追及するため，糞便及び乳製品の自然含有例について，また実験的に両者の菌を接種した滅菌脱脂乳の材料について，これらを種々の条件下に保存して両菌群の生残試験を行った．

I 実験材料及び方法

糞便材料は(IV)に述べた人及び各種動物の菌検索を終了した生理食塩水による10倍稀釈液46例である．これらを室温（年間を通じて大約5～25°C）に放置して3ヵ月毎に腸球菌群並びに大腸菌群の生残の有無を追及した．これらの検査材料は綿栓した中試験管内に保存され，蒸発乾固を防ぐため時々滅菌蒸溜水が注入された．両菌群に対する生残の確認は腸球菌群は既述の推定培地 No. 1, 2 及び確認培地により，大腸菌群は B.G.L.B. 醱酵管及び E.M.B. 培地により確認試験まで行って判定した．

乳製品のうちバターは Table 10 に示されたもののうち生菌数が g 当り5万以上のもの7例，2.8万のもの1例である．これらを4°C の冷蔵庫に保存しながら2ヵ月毎に取出して糞便材料同様に両菌群の変化を観察した．脱脂粉乳は既報[17]で述べた昭和30年度検査分の材料8例である．ポリエチレン袋入りの検体を室温に保存しながら3ヵ月毎に腸球菌群の変化を追及した．

次に食品中における腸球菌群並びに大腸菌群は保存温度を異にしたとき，時日の経過とともにどのような消長を示すかをみるため，上述の自然例に対して実験的に両者の菌を滅菌脱脂乳へ接種して観察を行った．まず *Str. faecium* 及び *E. coli* の各1株をそれぞれブイヨンに37°C 18時間培養後，単独あるいは混合して滅菌脱脂乳に加え，22°C で16時間増殖させた後，これらを4°C，室温及び37°C の3群に分けて保存しながら1月毎に両者の菌の消長を調べた．単独接種例は各温度2例づつ計12例，混合接種例は各温度1例づつ計3例，合計15例である．

II 実 験 成 績

1. 糞便における成績

実験開始時における腸球菌群数並びに大腸菌群数は Table 20 に示されたとおり，腸球菌群数は g 当り <12～2,700,000,000，大腸菌群数は <23～1,300,000,000 の範囲にあった．実験開始時において大腸菌群数が腸球菌群数より多かったもの29例についての成績が Table 38 に示される．本表において保存3ヵ月第1回目の試験で何れかの菌群が既に死滅しているものは No. 23, 30, 48 及び 55の4例，第2回6ヵ月目で両者の菌群とも死滅したものは No. 4, 5, 19 の3例，39ヵ月目でなお生残して試験続行中（→印）のものは11例である．

Table 38. Longevity of enterococcus group and coliforms in human and various animal feces preserved in room temperature (5～25°C)
(Twenty nine samples which the number of coliform bacteria was greater than the one of enterococcus group bacteria at the beginning of examination).

| Sample No. | Origin of feces | Months elapsed | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beginning | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 37 | 30 | 33 | 36 | 39 |
| 2 | Cattle | ⊜ | ⊜ | ⊜ | — | | | | | | | | | | |
| 3 | 〃 | ⊜ | ⊜ | ⊜ | — | | | | | | | | | | |
| 4 | 〃 | ⊜ | ⊜ | — | | | | | | | | | | | |
| 5 | 〃 | ⊜ | ⊜ | — | | | | | | | | | | | |
| 7 | Horse | ⊜ | ⊜ | ⊜ | △ | — | | | | | | | | | |
| 9 | 〃 | ⊜ | ⊜ | △ | △ | — | | | | | | | | | |
| 10 | 〃 | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ○ | ○ | ○ | — | | | | | |
| 13 | Sheep | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | △ | △ | △ | △ | △ | △ | — | | |
| 14 | 〃 | ⊜ | ⊜ | △ | △ | — | | | | | | | | | |
| 15 | 〃 | ⊜ | ⊜ | △ | △ | — | | | | | | | | | |
| 17 | Hog | ⊜ | ⊜ | △ | — | | | | | | | | | | |
| 18 | 〃 | ⊜ | ⊜ | △ | — | | | | | | | | | | |
| 19 | 〃 | ⊜ | ⊜ | — | | | | | | | | | | | |
| 20 | 〃 | ⊜ | ⊜ | ○ | — | | | | | | | | | | |
| 21 | Dog | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | △ | △ | △ | — | | | | |
| 22 | 〃 | ⊜ | ⊜ | ⊜ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | → |
| 23 | 〃 | ⊜ | △ | △ | △ | — | | | | | | | | | |
| 24 | 〃 | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | → | | | |
| 29 | Cat | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | △ | △ | △ | → | | | |
| 30 | 〃 | ⊜ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | → | | | |
| 39 | Rat | ⊜ | ⊜ | △ | △ | △ | — | | | | | | | | |
| 41 | Rabbit | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | → | | |
| 44 | 〃 | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | △ | △ | △ | → | | | |
| 48 | Human (Suckling baby) | ⊜ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | → |
| 50 | 〃 ( 〃 〃 ) | ⊜ | ⊜ | ⊜ | ○ | ○ | — | | | | | | | | |
| 51 | 〃 (Adult) | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | → | |
| 53 | 〃 ( 〃 ) | ⊜ | ⊜ | ⊜ | ⊜ | △ | △ | △ | △ | △ | △ | △ | △ | → | |
| 54 | 〃 ( 〃 ) | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | ⊜ | → | | |
| 55 | 〃 ( 〃 ) | ⊜ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | → | | |

Notes. ⊜ : Both enterococcus group and coliforms survive.
△ : Only enterococcus group survives.
○ : Only coliforms survives.
— : Both enterococcus group and coliforms are negative.
→ : Survival test is continued.

腸球菌群並びに大腸菌群の両者が同時に死滅しているものは No. 2, 3, 4. 5 及び19の 5 例，両菌群ともなお生残して試験続行中は No. 24, 41, 51 及び 54の 4 例である．既に死滅あるいはなお生残して試験続行中の何れにせよ，腸球菌群が大腸菌群より長く生残している例は No. 7, 9, 13, ……等の15例，これに反して大腸菌群の方が長く生残している例は No. 10, 20, 30, 50 及び 55のわずか 5 例に過ぎない．本表における材料はすべて保存開始時において大腸

菌群数の方が腸球菌群数より多い材料であったにも拘らず，保存による生残試験の結果は以上の如く，腸球菌群の方が長く生残する例が多い結果となった．

次に保存開始時において腸球菌群数の方が大腸菌群数より多かった17例についての成績はTable 39 に示すとおりである．保存 3 ヵ月目第 1 回検査時において，No. 27, 28 は既に両菌群とも死滅していた．39ヵ月目でなお生残して試験続行中は No. 25, 46 及び49の 3 例である．本表において腸球菌群が大腸菌群より長く生残しているのは14例であり，これに反して大腸菌群の方が長く生残しているのは，No. 43 のわずか 1 例であった．

Table 39. Longevity of enterococcus group and coliforms in human and various animal feces preserved in room temperature (5～25°C)
(Seventeen samples which the number of enterococcus group bacteria was greater than the one of coliform bacteria at the beginning of examination).

| Sample No. | Origin of feces | Months elapsed | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beginning | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 |
| 8 | Horse | ◎ | ◎ | △ | △ | — | | | | | | | | | |
| 25 | Dog | ◎ | ◎ | ◎ | △ | △ | △ | △ | △ | △ | △ | → | | | |
| 26 | Cat | ◎ | △ | △ | △ | △ | — | | | | | | | | |
| 27 | 〃 | ◎ | — | | | | | | | | | | | | |
| 28 | 〃 | ◎ | — | | | | | | | | | | | | |
| 33 | Fowl | ◎ | ◎ | ◎ | △ | — | | | | | | | | | |
| 34 | 〃 | ◎ | ◎ | △ | △ | △ | △ | △ | — | | | | | | |
| 35 | 〃 | ◎ | ◎ | △ | △ | △ | △ | △ | △ | △ | — | | | | |
| 36 | Rat | ◎ | ◎ | △ | △ | — | | | | | | | | | |
| 37 | 〃 | ◎ | ◎ | △ | △ | △ | △ | — | | | | | | | |
| 38 | 〃 | ◎ | ◎ | △ | △ | △ | △ | △ | △ | — | | | | | |
| 40 | 〃 | ◎ | ◎ | ◎ | △ | △ | △ | △ | △ | △ | △ | — | | | |
| 43 | Rabbit | ◎ | ◎ | ◎ | ○ | ○ | — | | | | | | | | |
| 46 | Human (Suckling baby) | ◎ | ◎ | ◎ | ◎ | ◎ | ◎ | △ | △ | △ | △ | △ | △ | △ | → |
| 47 | 〃 ( 〃 〃 ) | ◎ | ◎ | ◎ | ◎ | △ | △ | △ | — | | | | | | |
| 49 | 〃 ( 〃 〃 ) | ◎ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ | → |
| 52 | 〃 (Adult) | ◎ | ◎ | ◎ | ◎ | ◎ | ◎ | ◎ | ◎ | △ | — | | | | |

Note. Signs employed in this table are those in table 38.

以上の如く本表における検査材料は実験開始時，すでに腸球菌群数の方が大腸菌群数より多く存在していた材料であったためか，前表の材料に比べて更に腸球菌群の方が大腸菌群より長く生残した例が多数を占めた．

2. バター及び脱脂粉乳における成績

4°C の冷蔵庫に保存しながら 2 ヵ月毎に取出して検査したバターについての成績は Table 40 に示すとおりである．大腸菌群は陽性材料の 5 例中，No. 28 では製造後 4ヵ月以内，No. 31, 36 では 7ヵ月以内に消失し，No. 32, 35 は前 3 例よりも含有菌数が多いためか 7ヵ月目でなお生残していた．腸球菌群の減少も大体含有菌数に比例し，菌数の少ない No. 33 では 4 ヵ月以内，No. 29 は 5 ヵ月以内，No. 26, 28 は 6 ヵ月以内で消失し，比較的菌数の多かった No. 31, 32, 35 及び 36では 7ヵ月目でなお生残を示した．すなわち No. 28, 36 の如く大腸菌群数の方が多い材料にあっても腸球菌群の方が長く生残することが観察された．

Table 40. Longevity of enterococcus group and coliforms in butter preserved at 4°C.

| Sample No. | Months elapsed since manufacture at the first examination | Results every 2 months | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 28 | 0 | $1.1\times10^2$<br>*$3.0\times10^2$ | +<br>+ | +<br>− | −<br>− |
| 31 | 1 | $4.0\times10^2$<br>*$3.0\times10$ | +<br>+ | +<br>+ | +<br>− |
| 36 | 1? | $5.5\times10$<br>*$1.0\times10^2$ | +<br>+ | +<br>+ | +<br>− |
| 32 | 1 | $1.7\times10^3$<br>*$9.0\times10^3$ | +<br>+ | +<br>+ | +<br>+ |
| 35 | 1? | $6.5\times10^2$<br>*$9.3\times10^3$ | +<br>+ | +<br>+ | +<br>+ |
| 29 | 1 | $1.0\times10$ | + | − | − |
| 26 | 2 | $2.3\times10$ | + | − | − |
| 33 | 2? | $2.3\times10$ | − | − | − |

Notes. ? : Presumptive months elapsed.
* : Coliforms.
+ : Survival.
− : Death.

粉乳に含まれる生菌数が保存の経過に伴って減少する事実は既に報告[19)20)]したが，今回の脱脂粉乳における観察は腸球菌群数のみについて行った．なお大腸菌群の観察は本菌群が粉乳から検出される例が近年の製品では皆無のため出来なかった．成績は Table 41 に示されるように，含有菌数は一般に上述のバター例より多く，生存期間も長い傾向にあり，観察期間中死滅した例は No. 3 及び No. 5 の 2 例に過ぎず，しかもこれらの例も製造後15～17ヵ月間はなお生残していた．

Table 41. Longevity of enterococcus group in skim milk powder preserved in room temperature (5～25°C).

| Sample No. | Months elapsed since manufacture at the first examination | Results every 3 months | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 7 | $5.0\times10^3$ | + | + | + | + | + | + |
| 2 | 5 | $7.5\times10^3$ | + | + | + | + | + | + |
| 3 | 5 | $1.5\times10^4$ | + | + | + | + | − | − |
| 4 | 4 | $2.3\times10^4$ | + | + | + | + | + | + |
| 5 | 3 | $5.3\times10^4$ | + | + | + | + | − | − |
| 6 | 2 | $6.1\times10^4$ | + | + | + | + | + | + |
| 7 | 6 | $6.5\times10^4$ | + | + | + | + | + | + |
| 8 | 4 | $3.2\times10^6$ | + | + | + | + | + | + |

Notes. + : Survival.
− : Death.

### 3. 保存温度を異にした人工接種脱脂乳における成績

*Str. faecium* 及び *E. coli* を単独あるいは混合して実験的に滅菌脱脂乳に接種し22°Cで16

時間培養後，これらを4°C，室温及び37°Cの3群に分けて保存しながら1月毎に両者の菌の消長を追及した．その成績はTable 42に示される．

Table 42. Changes of number of *Str. faecium* and *E. coli* cultured in sterilized skim milk during their preservation at different temperature.

| Species | Preserved at | Number of organisms per ml | | | | |
|---|---|---|---|---|---|---|
| | | At beginning | After 1 month | After 2 months | After 3 months | After 4 months |
| F | 4°C | $1.1\times10^{7}$ | $1.6\times10^{8}$ | $2.2\times10^{8}$ | $3.3\times10^{8}$ | $3.2\times10^{8}$ |
| | R.T. | 〃 | $5.5\times10^{8}$ | $4.6\times10^{8}$ | $6.5\times10^{8}$ | $4.6\times10^{8}$ |
| | 37°C | 〃 | $3.2\times10^{5}$ | 0 | 0 | 0 |
| | 4°C | $1.0\times10^{8}$ | $2.9\times10^{8}$ | $3.5\times10^{8}$ | $3.2\times10^{8}$ | $3.1\times10^{8}$ |
| | R.T. | 〃 | $6.5\times10^{8}$ | $6.2\times10^{8}$ | $6.0\times10^{8}$ | $4.8\times10^{8}$ |
| | 37°C | 〃 | $2.1\times10^{5}$ | $5.5\times10^{4}$ | $2.3\times10^{5}$ | 0 |
| C | 4°C | $1.3\times10^{8}$ | $2.3\times10^{8}$ | $8.9\times10^{7}$ | $2.7\times10^{6}$ | $9.7\times10^{6}$ |
| | R.T. | 〃 | $8.7\times10^{4}$ | $1.6\times10^{2}$ | 0 | 0 |
| | 37°C | 〃 | 0 | 0 | 0 | 0 |
| | 4°C | $4.0\times10^{8}$ | $3.3\times10^{8}$ | $1.1\times10^{8}$ | $1.4\times10^{7}$ | $4.9\times10^{6}$ |
| | R.T. | 〃 | $4.9\times10^{6}$ | $1.3\times10^{3}$ | 0 | 0 |
| | 37°C | 〃 | 0 | 0 | 0 | 0 |
| F+C | 4°C | $2.9\times10^{7}$ | $4.4\times10^{7}$ | $8.0\times10^{6}$ | $1.6\times10^{7}$ | |
| F | | $2.6\times10^{6}$ | $4.0\times10^{7}$ | $6.7\times10^{6}$ | $1.2\times10^{7}$ | |
| C | | $2.6\times10^{7}$ | $3.9\times10^{6}$ | $1.3\times10^{6}$ | $3.8\times10^{6}$ | |
| F+C | R.T. | $2.9\times10^{7}$ | $1.9\times10^{8}$ | $2.4\times10^{8}$ | $2.2\times10^{8}$ | |
| F | | $2.6\times10^{6}$ | $1.9\times10^{8}$ | $2.4\times10^{8}$ | $2.2\times10^{8}$ | |
| C | | $2.6\times10^{7}$ | $8.0\times10^{4}$ | 0 | 0 | |
| F+C | 37°C | $2.9\times10^{7}$ | $7.3\times10^{5}$ | $6.0\times10^{3}$ | 0 | |
| F | | $2.6\times10^{6}$ | $7.3\times10^{5}$ | $6.0\times10^{3}$ | 0 | |
| C | | $2.6\times10^{7}$ | 0 | 0 | 0 | |

Notes. F : *Str. faecium.*
C : *E. coli.*
R.T. : Room temperature (5～25°C).

22°C 16時間培養で腸球菌は最大値まで発育増殖出来なかったためか，4°C並びに室温保存ではその後も増数を続け，保存後2～3カ月目で漸く最大値に達している例もみられる．しかし大腸菌は22°C 16時間培養でほぼ最大値に達している模様である．

接種菌の消長は，単独接種例では，4°Cにおいて両種菌とも，室温では腸球菌が4カ月目でなお生残を示したが，大腸菌は3カ月目で既に死滅し，37°Cでは腸球菌が2～4カ月，大腸菌は1カ月目で死滅するのが認められた．混合接種例において，保存開始時における大腸菌数は腸球菌数の10倍も存在したにも拘らず単独接種例同様大腸菌の方が早く死滅した．以上人工接種脱脂乳においては大腸菌の死滅は腸球菌よりも早く，また保存温度が高い程両種菌ともすみやかに死滅した．

### III 小 括

人及び各種動物の糞便稀釈材料を室温に長期間保存して腸球菌群並びに大腸菌群の生残状態を追及した結果，保存開始時において腸球菌群数の方が大腸菌群数より多い例は勿論，少ない例においても腸球菌群の方が大腸菌群より長く生残する例の多いことが認められた．また冷蔵庫に保存したバターの例においても糞便の例同様一般に腸球菌群の方が長く生残した．一方脱脂粉乳を室温に放置してその中に含まれる腸球菌群の生存期間を検討した結果，製造後少なくとも15～17カ月以上生残することを知った．また *Str. faecium* 並びに *E. coli* を実験的に滅菌脱脂乳に接種し，保存温度を4°C，室温及び37°Cの3段階に分けて両者の菌の消長を観察した結果，*E. coli* の死滅は *Str. faecium* より早く，また保存温度が高い程両種菌の死滅がすみやかであった．

## (VIII) 考 察

汚染指標菌としての腸球菌群に関する研究はその選択培地の改良進歩と相俟って進んで来た．緒言において述べた如く，腸球菌群の選択培地は多くの研究者の努力により，近年著しく改良が加えられて来たが，基本は他種菌に対する制菌剤としての Sodium azide の使用であり，更に Ethyl violet の添加あるいは45°C培養法との併用である．しかし Sodium azide の濃度は報告者によって一定しておらず，各研究者が独自の処方をもって最良の培地と推奨しているのが現状である．

著者ら[21]は B.T.B. Azide Dextrose Broth を用い37°Cと45°Cにおける腸球菌群の発育を比較した結果，37°Cに比べて45°C培養における発育が著しく阻害されたことから，腸球菌群の定量的検査に当っては45°C培養が不適当であることを既に報告した．また乳製品の如き加熱材料にあっては，推定培地の Sodium azide の濃度が0.02%で十分であったが，生乳，生肉等の非加熱材料の如く，腸球菌群以外の他種菌が圧倒的に多い材料では，腸球菌群以外のレンサ球菌あるいはブドウ球菌等の発育がみられ選択能力に不十分な点があることを知ったので，まず今回は主として Sodium azide の濃度を増すことと，Ethyl violet の併用について若干の検討を試みた次第である．

腸球菌群選択培地として初期に報告された HAJNA & PERRY[16]の S. F. 培地には Sodium azide が高濃度（0.05%）に含まれ，更に培養温度は45°C を採用していることから，腸球菌群そのものも非常に発育を阻害され定量的検査には不適当であることが MALLMANN & SELIGMANN (1950)[47]によって既に指摘されている．一方 Sodium azide を低濃度（0.02%）で使用した推定培地については LITSKY ら[44]，堀江[26]及び那須[55]の報告があり，LITSKY らの培地では腸球菌群以外のグラム陽性球菌と桿菌が発育するため，確認培地の段階において Sodium azide を0.04%とし，更に Ethyl violet を添加することによって検査の確実を期している．堀江は LITSKY らの培地を改良して，確認培地の段階では Sodium azide の濃度を0.025%とし，更にCHILDS & ALLEN (1953)[12]の報告を参考として45°Cにおける培養法を組合せることによって他種菌の発育を抑制し，ほぼ満足すべき成績を得たと報告している．那須の培地は著者らによる検討の結果，上述の如く不十分な点が認められている．さて推定培地について検討した結果は Table 3 に示されたように，培地 No. 1 (Sodium azide 0.02%) では腸球菌群以外の他種菌の発育することが認められた．培地 No.2 (Sodium azide 0.025%) においてもやはり発育する他種菌はあったが，同濃度に Sodium azide を含む確認培地への

培養では *Str. bovis* の発育が不十分ながら認められた他は他種菌の発育が抑制され，またたとえ発育するものがあっても集落が黄変するまでに至らなかったことから，Sodium azide は0.025%の濃度で十分選択能力が発揮されることを知った．また Ethyl violet を特に添加する必要性は認められなかった．

次に培地 No. 2 においても発育を示した *Str. bovis* 及び腸球菌群を供試菌として，微量な菌を接種した場合の発育性を各推定培地で比較検討したところ，Table 4 に示されたように腸球菌群は培地 No. 1 においても対照培地の 4 %減，培地 No. 2 で 8%減，Ethyl violet 添加の培地 No. 4 では15%減の成績を得，*Str. bovis* は更にこれ以上の抑制を受けた．これらの成績から Sodium azide の濃度は検査に支障がない限り，出来るだけ低濃度にすることが必要であることを改めて確認した．しかし多種多様にわたる食品材料については，単一の培地を使用するよりも大腸菌群の検査における乳糖ブイヨンと B.G.L.B 培地の使い分けの如く，検体によって腸球菌群検索の培地も変えることが，検査を容易にするため必要であると考えるに至った．すなわち生乳，生肉等の如く出現する菌が多種多様でしかも菌数が多い検体では培地 No. 2 を，乳製品の如く加熱工程を経る結果，出現菌の傾向がほぼ一定し，かつ菌数の少ない検体では培地 No. 1 で十分である．但し Sodium azide の濃度を高めて使用するときは，それだけ腸球菌群自身も発育抑制の影響を受けて実際の菌数よりも少なく出現することを考慮すべきである．なお改訂された食品衛生検査指針 I (1959)[39]には腸球菌群検査用培地が記載されており，Sodium azide の濃度が 0.04%となっているが，以上述べて来た理由により，腸球菌群が多量に存在する検体について定性試験を行うのならば兎も角，腸球菌群の含有菌数が極めて少ない検体についての定量的試験を行うためには Sodium azide の濃度が高過ぎることは明らかであり改訂が望まれる．

畜産食品における腸球菌群の分布成績は Table 5, 6, 10, 11, 12, 15, 17 に示されたように，プラント持込乳，生肉（夏季）において腸球菌群は100%陽性であった．これらはまた大腸菌群も 100% 陽性であり，しかも大腸菌群の出現菌数の方が大であった（Table 7, 16参照）．冬季検査の生肉では腸球菌群は62%，大腸菌群は82%の割に陽性で本検体もその大部分において菌数は大腸菌群の方が大であった（Table 13参照）．しかるに製品においては腸球菌群の陽性率の方が高い成績を示した．粉乳（1958年度検査分）において腸球菌群は全材料から検出されたのに対し，大腸菌群は 1 例も検出されなかった．八田ら[22]は市乳の検査においてこれと全く同様な結果を得たと報告している．なお畜産食品あるいは一般食品についてのその他の報告をみると，Wilson ら[86][87]はカキ，那須[56]はイカ，タコ，宮林[51]は生肉，貝類，サラダ，市川・国田[29]は生肉，堀江[27]は鮮魚介類等の非加熱食品においては一般に大腸菌群の陽性率が腸球菌群のそれより高く，また菌数も多いと述べている，一方村磯[54]は生菓子の餡，宮林[51]はコロッケ，シュウクリーム，青木ら[1] は粉乳，ミックスパウダー，市川・国田は肉製品等の如き加熱食品においては腸球菌群が大腸菌群に比べて陽性率が高いと報告しているが，耐熱性の弱い大腸菌群が死滅し，より耐熱性を有する腸球菌群が生残するのは当然のことと考えられる．今回検査した畜産食品において上述の報告同様，生乳，生肉材料にあっては大腸菌群の陽性率が，乳製品にあっては腸球菌群陽性率が高い成績を示した．但し製造工程中に加熱されると思われる肉製品においては，やはり加熱を受ける乳製品同様の成績を得られなかったが，これは製造方法，製品の性質，形態も異なり，また保存料その他の添加等によって影響を受けたためかも知れない．更に現今のわが国における肉製品の製造状態をみるとき，乳製品製造におけるような高度の衛生水準にないものが多いと想像されるため，種

々の条件により加熱材料たるにも拘らず非加熱材料の如き結果が得られたのではなかろうかとも考えられる．

村磯，宮林は上述のような腸球菌群の検出率の高い食品にあっては，大腸菌群よりもむしろ腸球菌群を汚染指標菌として検索する方が，食品の安全性をより高めるという点で，また加熱前における原料の汚染状況を推測出来る点において優れているのではなかろうかと述べているが，このことは汚染指標菌の条件の一つとして重要なことと思われる．

次に腸球菌群，大腸菌群及び一般生菌数三者の出現状態を検討すると Table 19 に示された如く，プラント持込乳及び夏季生肉材料では腸球菌群も大腸菌群も全例陽性であり，生菌数もすべてg当り5万以上から1,100万以上にまで分布していた．冬季生肉材料では両菌群ともその検出率は100%に達せず，生菌数も幾分少なく全例の32%は5万以下であったが，その他は400万以下あるいは1,100万以上にまで分布していた．加工品において粉乳では両年度分とも腸球菌群のみ陽性であり，大腸菌群は陰性，生菌数もすべて5万以下であった．小メーカーのバター及び肉製品においては大腸菌群が陽性であったが，これらには生菌数5万以上の例が存在した．以上のことから生菌数と腸球菌群あるいは大腸菌群との関係を考察すると，大腸菌群の方が腸球菌群よりも生菌数の多い材料と，より関連性があるように見受けられる．事実バターにおいて大腸菌群陽性の5例はすべて生菌数も5万以上の材料であった．しかし生菌数の多い材料が必ずしもすべて大腸菌群陽性とは限らず，個々の材料について観察すると，腸球菌群陽性例と生菌数の多い例とが関連している場合も多い．結局腸球菌群，大腸菌群及び生菌数三者の検査を行い総合的に判定することが，最も汚染を確実に知る方法と考えられる．宮林[50]は食品汚染の状況を判定するため腸球菌群を検査するには，大腸菌群の検査もあわせて行い，両者の成績を比較して総合的に判定することが必要と述べ，Buttiaux[11]は大腸菌群と腸球菌群の両者が存在した検体では糞便による汚染が確実に存在したことを意味すると述べていることはこの間の事情を物語っている．

乳及び乳製品の成分規格等に関する省令には生菌数及び大腸菌群についての成分規格が明確に定められている．本省令が公布された当時の主旨は，乳幼児にとって必需品である乳及び乳製品の品質の衛生管理を特に一般食品よりも厳格に行う必要があると認めたからであり，品目の範囲も限定されていた．最近一部改正が行われてバター及びチーズ等2，3が追加されて品目の範囲も拡大されたが，乳及び乳製品が乳幼児の必需品であることに変りはなく，更に老幼病弱者の蛋白補給源として重要な位置を占めつつある今日，このような食品については衛生保持を如何に厳格に行っても行い過ぎるということはなく，三者の出現状態からこれら製品の安全性を高めることは必要欠くべからざるものと考えられる．

乳製品のうち煉乳，プロセスチーズでは腸球菌群も大腸菌群も陰性であった．煉乳について腸球菌群を調べた報告は見られないが，プロセスチーズについては大嶋[65]の報告があり，腸球菌群は生チーズでは陽性であったが製品では陰性であったと述べていることから，これらの製品には普通腸球菌群がみられない材料と考えてよいかも知れない．換言するとこれらの製品から腸球菌群が検出されたときは，殺菌が不十分であったか，殺菌後の汚染があったと考えられる訳である．

汚染指標菌としての見地から調べられた水，土壌あるいは食品等における腸球菌群の分布に関しても数多くの報告がみられる．一方その汚染源としての人あるいは各種動物の糞便における腸球菌群の分布に関しても数多くの報告がなされている．Smith & Sherman (1938)[83]は人の糞便について腸球菌群を検索しているが，分離に際しては未だ腸球菌群の選択培地

が用いられていない．1946年 WINTER & SANDHOLZER[89] は WHITE & SHERMAN[85] の培地を改変した選択培地を用いて人，家畜及び野獣の腸球菌群並びに大腸菌群を調べて両菌群の菌数を比較し，大腸菌群数は比較的一定しているが，腸球菌群数は分布の巾が大であると報告した．

従来糞便中に含まれる腸球菌群の菌数は一般に大腸菌群数の 1/10 を越えることは稀であるとされていた．それは現在の如き良好な腸球菌群の選択培地がなかったためでもある．しかし腸球菌群の選択培地を用いて行った人及び各種動物の糞便検査成績[9)23)27)67)] によれば，やはり大腸菌群数の方が腸球菌群数より大であったと報告している．SLANETZ & BARTLEY[82] は Membrane filter 法により，人，ウシ及びその他の動物の糞便40例について両菌群の菌数を比較した結果，腸球菌群の方が大であったと報告しているが，これは SLANETZ らがいわゆる腸球菌群と称している菌群の範囲を拡大して *Str. bovis* 及び *Str. equinus* 等をも含めた際の成績である．さて今回著者が行った人及び各種動物55例の検査結果は，Table 20 に示したように両菌群とも 100% 陽性であったが，菌数の比較では上述の報告同様大腸菌群数の方が腸球菌群数よりも多い例が多数を占めていた (Table 22 参照)．しかしニワトリ，ネズミ，初生児では逆に腸球菌群数の方が大腸菌群数より多い成績を示した．ネズミの糞便において腸球菌群の菌数あるいは検出率が大腸菌群のそれらより大であるとの報告は OSTROLENK & HUNTER[67],佐久間[73] によってなされており，また青木[2] は溶血性腸球菌の検出率が高かったと報告している．各種伝染病あるいは食中毒菌の媒介者であるネズミにおいて腸球菌群の菌数あるいは検出率が大腸菌群のそれらよりも大であったという事実は，食品の汚染状態を調べるため腸球菌群を指標菌として使用する意義を高めるものである．

プラント持込乳，粉乳及び生肉等において腸球菌群は 100% 陽性を示していたが，これらの汚染源調査という意味で，人及びウシの糞便を特に取上げて，しかも腸球菌群のみを対象として検索を行った．成績は Table 23, 24 に示された如く，人50例では全例陽性であった．BUTTIAUX[9] はウシ，ヒツジの糞便では腸球菌群の陰性例も認められたと報告し，青木[2] も各種動物の糞便を検査して相当数の陰性成績を得，特にウシではわずか 7% (2/28) の検出率であったと報告しているが，著者のウシ 30例の成績は 100% 陽性であった．ウシにおける出現菌数は $10\sim10^4$ であり，人の $10\sim10^7$ に比べ分布の巾も小である．腸内細菌叢が食餌成分によって影響を受けることは想像されるところであり，これらの関係については大嶋[64)66)] の報告にも詳しいが，腸球菌群もその影響を受けているであろうことは論を俟たない．ウシにおいて各牛舎毎の出現菌数が一定した傾向を示したことは，環境を同じくする上に同一飼料の給飼等が大きく影響していたためと考えられる．人において分布の巾が大であったのはウシと全く逆の理由によったものであろう．人及び各種動物各5例の腸球菌群の平均出現菌数は Text-fig. 1 に示したとおりであるが，ウシにおける腸球菌群の出現菌数は11種の材料中最低の成績を示している．しかしこの成績は後から行われた 30例の出現菌数とほぼ同じ成績であった．なお11種の糞便材料中腸球菌群の出現菌数が最大を示したのはイヌにおいてであるが，大嶋[66] もウシ，ウマ，ヒツジ，ヤギ，ブタ及びイヌの6種の糞便を調べてイヌにおける菌数が最大であったと報告している．我国においてイヌが各種 *Salmonella* の有力な媒介者であること[59)60)72)]，更に *Salmonella* の中には食中毒の原因菌となり得るものが多数あることは周知の事実であるが，このようなイヌの糞便において腸球菌群並びに大腸菌群がともに最高に存したということは，両者の菌群がともに汚染指標菌として有用であることを物語る一面である．

さて大腸菌群の汚染指標菌としての価値が低いといわれる理由として本菌群の中には糞便由来以外の自然界に常在する *A. aerogenes* (*Klebsiella*) 等が含まれているため，大腸菌群が検出されたとしても真の糞便による汚染かどうかは疑わしいという意味のことを緒言において述べた．しかしこの説に対しては今日大腸菌群を更に IMViC test による型別を行うことにより，土壌由来といわれている *A. aerogenes* と真の糞便由来といわれている *E. coli* とを区別出来ることから，*E. coli* の検出を目標とすれば，汚染指標菌としての価値はすたれないとの反論がある，ここにおいて BUTTIAUX[11] は最近数年間における人の糞便内大腸菌群を調査した結果，近年の抗性物質使用の影響によって，腸内菌叢に変化が起り，糞便内における *E. coli* が減少し，代って *A. aerogenes* が増加して来たと述べ，従来の *E. coli* は糞便由来，*A. aerogenes* は主として土壌由来という説に批判を加えている．更にD群レンサ球菌は人あるいは動物の糞便内において大腸菌群と同じかあるいはそれ以上に出現する例も多いことから汚染指標菌としての価値が増大して来たと報告しているのは興味深い．

腸球菌群の分類に関しては未だ種々の異論があるので，著者は Bergey's Manual[8], SEELEMANN[74] の Biologie der Streptokokken, その他[4)75)76)80)] の報告 を参考として分類を試みた．レンサ球菌の分類に関する研究は我国にも多く，小林一門，越智らその他の報告があり，越智ら[62] の分類によれば，レンサ球菌は熱抵抗性（60°C 30分）及び胆汁抵抗性（40%）によって3群に分けられ，腸球菌群は両者の抵抗性を有するものとされているが，SEELEMANN その他との関連性については不明である．

さて畜産食品から分離したレンサ球菌592株，糞便から分離したレンサ球菌376株計968株について各種抵抗性試験，糖分解能，Potassium tellurite に対する抵抗性及び TTC の還元性，Lancefield によるD群の血清沈降反応及びその他の生物学的性状の検査を行い，それらの成績を総合的に判定した結果，腸球菌群873株，腸球菌群以外のレンサ球菌95株に分けられた．腸球菌群の内訳は Table 30 に示されたように *Str. faecalis* 101株，*Str. faecalis* var. *liquefaciens* 100株，*Str. faecalis* var. *zymogenes* 3 株，*Str. durans* 93株，*Str. faecium* 438株，Unidentified enterococci 138株であり．腸球菌群以外のレンサ球菌は *Str. bovis* 21株 及び不明レンサ球菌74株であった．

上述の如く腸球菌群を分類した結果 *Str. faecium* は腸球菌群合計873株中の50%を占める成績であった．我国における食品あるいは糞便から分離された腸球菌群の分類に関する報告は Bergey's Manual に従っているものが多いため，*Str. faecium* を独立の種として型別した報告は見当らない．堀江[24] は糖分解能の成績から腸球菌群を I, II, III型に分け I 型が ORLA-JENSEN の *Str. faecium* に相当するものであると述べているが，Potassium tellurite に対する抵抗性及び TTC の還元性試験を欠除しており，従って SKADHAUGE[80], BARNES[4], その他[74)75)76)] の報告との関連性までは言及していない．*Str. faecium* は特に Potassium tellurite 及び TTC に対する感受性の差から型別されることを既に述べたが，更に他の主要な鑑別点として Arabinose を分解することが必要条件[3)4)31)35)76)] として挙げられている．しかし SEELEMANN[74], MOUTOUSSIS ら[52] は非分解株の例外もあると述べているから，この点を考慮すると著者が Unidentified enterococci として分けた138株の中には Atypical faecium も含まれていると想像される．かくすれば著者の検査株において *Str. faecium* の出現率は更に増大する訳で，腸球菌群の中において本菌種の占める比率及び価値はますます増大するものと考えられる．緒言において述べたように MUNDT ら[53], その他[9)10)42)88)] も *Str. faecium* が最も多く検出される型の腸球菌であると報告しているが，著者の成績も全くこれらと一致

した.

次に腸球菌群の同定分類成績に基づいて材料別に出現菌型を検討した結果，Table 32に示されたように，畜産食品において生乳，生肉等の非加熱材料では faecalis 系 (*Str. faecalis* 及びその varieties)が *Str. faecium* 及びその他(*Str. durans* 及び Unidentified enterococci) より多く，加熱材料の乳製品では *Str. faecium* の出現率が最も大であった.

一方糞便における成績をみると，人の糞便からは faecalis 系が検出されるが，ブタ，ウシ，ヒツジでは少なく，*Str. faecium* がその主要な菌であるといわれている[3)13)]. また BUTTIAUX[9)]は人，ブタ，ウシ及びヒツジの 糞便由来腸球菌群 を分類した結果，*Str. faecium* はすべての材料から多数検出されたが，faecalis 系はウシ，ヒツジに少なく人，ブタでは 多いと報告し，前者の報告とはブタの点で 異っていた. 更に WILSSENS & BUTTIAUX[88)] はウシの糞便37例を調べて 100%に *Str. faecium* を検出している. 著者の成績では Table 33 に示されたように，各種動物 及び ウシの糞便では *Str. faecium* が最も多く検出され，特にウシでは 96.6% の出現率であった. 人ではウシ及び各種動物と同様に *Str. faecium* が多数検出されたが，更に faecalis 系が多く検出された 点において 動物の糞便材料とは 異った 成績を示した.

以上から 糞便中の腸球菌群としては，一般に人においては faecalis 系と *Str. faecium* が，その他の動物特にウシにおいては *Str. faecium* のみがより多く存するようである. 人と動物由来の主要な腸球菌の型が異なるという点は，大腸菌群にみられない腸球菌群の特性と考えられ，著者の成績も以上の報告と同じような結果が得られた. これらの事実から検出された腸球菌群を型別することにより，人あるいは動物の糞便による汚染の違いをある程度区別することも可能と考えられる.

さて 非加熱材料に faecalis 系が，加熱材料に *Str. faecium* が多かったという事実は，これら菌種間の熱抵抗性に 差があったためでは なかろうかという疑問を 抱かしめた. よって *Str. faecalis*, *Str. faecalis* var. *liquefaciens* 及び *Str. faecium* の 3 菌型について浮遊液基質，感作温度，作用時間，接種菌数等の条件を考慮して熱抵抗性試験を行った. 接種菌数は出来るだけ同じになるよう注意を払ったが，検定の結果はすべて同じ母集団に属することが確められた($P<0.01$). 試験の成績は Table 36, 37に示されたように，*Str. faecium* の熱抵抗性が最も強く，*Str. faecalis* var. *liquefaciens* が最も弱く，*Str. faecalis* がその中間に位した. この試験結果は生材料では faecalis 系が，加熱材料では *Str. faecium* が多かったという成績を 裏書きするものである. 八田ら[22)]は *Str. faecalis* を供試菌として 菌数と温度とを種々組合わせて熱抵抗性試験を行った結果，株により差があることを認めているが，この実験において使用した *Str. faecalis* 株の中には *Str. faecium* 株もあったのではなかろうかと推定されるので，両者の型を区別していたならば，あるいは一定した傾向がみられたかも知れない. また IYENGARら[33)]は *Str. faecalis* 及び *Str. liquefaciens* を供試菌として 著者と同じような条件の下に熱抵抗性試験を行い，幾分 *Str. liquefaciens* の方が *Str. faecalis* より感受性があるような成績を 得ているが，著者の 成績にみられる如き 顕著な差を 得ていない. INGRAM & BARNES[3)31)]は 罐詰ハムから分離した腸球菌群 17株を同定した結果，*Str. faecalis* が認められず16株が *Str. faecium* であったという成績を得た. 更に ブタの腸管から得た腸球菌群の 同定の結果も *Str. faecium* が多く，*Str. faecalis* が認められなかったことから，罐詰ハムから分離された *Str. faecium* はブタ腸管由来のものであろうと推定した. 更に BARNESら[5)6)]は ベーコン工場における材料 及び種々の器具について 腸球菌群を調べた

結果では faecalis 系が *Str. faecium* 同様多かったことから，罐詰ハム製造の過程においても当然 faecalis 系と *Str. faecium* の汚染を受けて両者の菌が出現せねばならぬという結論に達した．しかし罐詰ハムから実際に検出されたのは *Str. faecium* のみであったことから，この理由は両者の菌の熱抵抗性の差あるいはハム中における両者の菌の発育の違いによるものかも知れないと推論したが，それ以上を明らかにしていない．しかし BARNES らが罐詰ハムの検査で *Str. faecium* のみが検出されたという成績は，著者の実験結果から，その製品以前の段階すなわち生肉原料では faecalis 系と *Str. faecium* の両方の汚染を受けていたことは明らかであり，この汚染された生肉原料が罐詰ハムに加工される工程中で加熱操作を受けるため熱抵抗性の弱い faecalis 系が死滅し，より耐熱性を有する *Str. faecium* のみが生残した結果と推定される．また KERELUK[35] は冷凍肉パイから分離した腸球菌群を同定した結果，ニワトリ，七面鳥，牛肉のパイでは，faecalis 系が多いが，罐詰のマグロを用いて作ったマグロのパイのみは *Str. faecium* が多かったと報告している．本例における出現菌型の差も罐詰ハムの例同様，加熱の影響によって *Str. faecium* のみが生残った罐詰のマグロをパイの原料としたため，マグロのパイのみに *Str. faecium* が多く出現したのであろう．すなわち同じ腸球菌であっても菌型により熱抵抗性に差があることから，加熱を伴う材料にあっては検出菌種に偏りがみられた訳である．このような腸球菌群の特異性を利用して加熱食品にあっては，出現腸球菌群を同定して faecalis 系か，*Str. faecium* かを型別することにより，その食品の加熱の程度，あるいは殺菌後の汚染状態をある程度推定出来るものと考えるに至った．

著者は更に 5 工場由来の粉乳 81材料から分離した 160株のレンサ球菌を分類し，更に糖分解能によって型別した成績に基づいて，工場別の出現菌型を検討した結果，Table 35 に示されたようにある特定の型の菌が工場別にしかも毎日連続して出現していることを知った．このことは工場所在地の地方に特定の型の菌が分布しているためか，あるいは粉乳製造過程のどこかで特定の型の菌による汚染を常時受けているのではないかとの疑いを生ぜしめる．しかし後者の場合 5 工場すべてにこのような現象が起きているとは考えられないから，やはり腸球菌群の分布が地方によって異る結果であろうと考えたい．このように工場によって出現する菌の型が異り，しかも特定の工場の製品から一定した型の菌が連続出現したということは，製品から分離した腸球菌群を型別することにより，どこの工場由来の製品であるかを推定出来る点で興味ある成績と思われる．

腸球菌群と大腸菌群の保存による生残試験において，糞便材料では Table 38, 39に示されたように保存後 3 年以上を経過してもなお生残している材料を含めて，供試材料46例中腸球菌群の方が長く生残したもの29例，それに反して大腸菌群の方が長く生残したもの 6 例，両菌群同じが11例で腸球菌群の方が長く生残することを知った．しかも46例中，試験開始時における両菌群の菌数は腸球菌群よりも大腸菌群の多い方が29例であったにも拘らずこのような成績が得られたのである．OSTROLENK ら[68] は糞便あるいは糞便で処理した土壌を保存して調べた結果，腸球菌群の方が長く生残したと述べているが，他方 MALLMANN & LITSKY[48] は汚水で処理した土壌では大腸菌群の方が長く生残したと述べ，更に堀江[28] は井戸水，海水を種々の条件で保存した結果も大腸菌群の方が長く生残する成績を得ている．これらの成績は試験材料に含まれている有機物の量と関係があり，腸球菌群の増殖が起らない程度の有機物の存在下でも大腸菌群の増殖が起り，菌数が増加するため生存期間も延長したような結果が得られたものと考えられる．*Str. faecium* と *E. coli* を実験的に接種した脱脂乳の保存試験

では Table 42 示されたように *Str. faecium* が *E. coli* より長く生残したが，これは上述の堀江の成績とは逆に22°C 16時間増殖後の保存開始時において，*E. coli* の発育増殖は殆んど最高にまで達していたが，*Str. faecium* は未だ最高の発育増殖までに至らず，なお保存中に増殖を続けたことも *Str. faecium* が長く生残した理由の一つと考えられる．またバターを4°Cの低温で保存したとき，大腸菌群数が腸球菌群数より多い材料にあっても腸球菌群の方が長く生残した(Table 40)．KERELUK ら[36]は両者の菌群を実験的に肉水に接種して-6°Fで保存したときは腸球菌群の死滅が大腸菌群のそれより低かったといい，堀江[28]もまた魚介類を実験的に汚水に浸漬させたものを-20°Cで長期間保存したとき，大腸菌群は漸次死滅する傾向が認められたのに対し，腸球菌群の減少はみられなかったと述べている．なおまた脱脂粉乳を室温に保存して含まれる腸球菌の消長を観察した成績は Table 41 に示されたように，20～25カ月後でも生残が認められた．

以上の如く著者の実験あるいは他の報告例においてもみられるように，種々の食品を種々の条件下に保存したとき，その中に含まれる大腸菌群の死滅は一般に腸球菌群よりもすみやかであった．乳肉製品の如き保存食品にあっては，製造直後には生残していたかも知れない大腸菌群も一定期間を経過した後では死滅し，腸球菌群のみが生残している場合が多いと考えられる．従ってこのような例では汚染指標としての大腸菌群のみの検査では判定が不満足な点もあるゆえ，腸球菌群の検査も同時に行うことが必要である．

## (IX) 総　　括

各種畜産食品に含まれる腸球菌群について，まずその推定培地を検討した後，これら食品における定性的並びに定量的検査を行ってその検出と分布状態を，また検出された菌の型別と検査材料との関係を検討し，更に検出菌の菌型の差による熱抵抗性試験及び腸球菌群の保存による生残試験を行った．一方汚染源としての人及び各種動物の糞便についても畜産食品におけると同様の調査研究を行って両者間の関係を考察した．更に大腸菌群，一般生菌数との関係をも考慮し，総合的に畜産食品に含まれる腸球菌群に関する衛生学的研究，特に汚染指標菌としての意義について検討を加えた．

以上の成績を要約すると次の如くである．

1. 選択培地を用いるときは，検査材料の汚染の程度に応じて培地を使い分けることが望ましい．但し Sodium azide の濃度が高まるにつれて腸球菌群自身の発育も抑制されることを考慮して使用すべきである．

2. 畜産食品の腸球菌群並びに大腸菌群の分布において，生食品では両菌群ともほとんど100%陽性であり，菌数の比較では大腸菌群の方が多かった．しかし加熱食品では腸球菌群の陽性率が高く，特に粉乳において大腸菌群は100%陰性にもかかわらず腸球菌群は100%陽性の成績であった．

3. 人60例，動物75例の糞便において腸球菌群は100%陽性であった．

4. 畜産食品及び糞便由来のレンサ球菌968株を同定した結果，*Str. faecalis* 101株，*Str. faecalis* var. *liquefaciens* 100株，*Str. faecalis* var. *zymogenes* 3株，*Str. durans* 93株，*Str. faecium* 438株，Unidentified enterococci 138株，*Str. bovis* 21株，不明レンサ球菌74株に分類された．なお *Str. durans* 及び *Str. faecium* の同定基準は Bergey's Manual のそれとは異なる．

5. 腸球菌群873株中 *Str. faecium* は最も多くその半数を占めた.

6. 畜産食品において食品別に検出菌型を検討した結果，生食品では *Str. faecalis* 及びその varieties の出現が *Str. faecium* より多く，加熱食品では *Str. faecium* の方が多かった.

7. 粉乳由来腸球菌群を糖分解能により型別した結果，特定工場製品から特定の型の菌が検出される傾向があった.

8. 人及び各種動物の糞便においては，個体別，種類別にみても大腸菌群数が腸球菌群数を上廻る例が多く，また人の糞便における腸球菌群数はウシの糞便におけるよりも遥かに多かった.

9. 糞便における検出菌型を比較検討すると，*Str. faecium* は動物特にウシの糞便において検出率が高く，人糞便においては *Str. faecalis* 及びその varieties と *Str. faecium* とは同程度に出現する傾向にあった.

10. *Str. faecalis*, *Str. faecalis* var. *liquefaciens* 及び *Str. faecium* の3菌型を対象として種々の条件下で熱抵抗性試験を行った結果，*Str. faecium* の耐熱性が最も強く，*Str. faecalis* var. *liquefaciens* が最も弱く，*Str. faecalis* が中間に位する成績を得た．本実験結果は生食品と加熱食品における出現菌型の差を実験的にも説明するものである．なお菌型を問わず含有菌数が少なければそれだけ死滅し易かった.

11. バター，糞便及び人工接種脱脂乳を室温その他の温度で保存して含まれる腸球菌群と大腸菌群の消長を追及した結果，何れも腸球菌群の方が長く生残した．このような現象は，試験開始時において大腸菌群数の方が多い例においても認められた．なお保存温度が高いほど両菌群の死滅は早い.

12. 室温保存の粉乳中の腸球菌群は，製造後15～17カ月以上においても生残した.

13. 乳肉及びそれらの製品については生菌数，大腸菌群とともに腸球菌群の検査もあわせて行い，三者の出現状態から総合的に汚染を判定することが食品の安全性をより高めるものである.

14. 乳製品の如く腸球菌群の出現率が大腸菌群よりも高い食品にあっては，大腸菌群よりもむしろ腸球菌群を汚染指標菌として検索する方が，食品の安全性をより高めるという目的にかなうものである.

15. 煉乳，プロセスチーズの如き製品から腸球菌群が検出される時は，加熱殺菌が不十分であったか，あるいは殺菌後の汚染が疑われる.

16. 保存食品にあっては，製造直後には存在したかも知れない大腸菌群が保存の経過に伴って死滅することから，保存による減少の少ない腸球菌群を汚染指標として調べる方が，製造前あるいは製造後の汚染状態を推測出来る点においても有利である.

17. 畜産食品において，生材料と加熱材料とにおける出現腸球菌群の菌型の差は，耐熱性の差によるものであることが実験的にも証明されたが，これらの成績から加熱材料においては出現する腸球菌群を型別することにより，加熱の程度もしくは殺菌後の汚染をある程度推定することが可能である.

18. 人及び各種動物の糞便から出現する腸球菌群の型には一定の傾向が認められるので，食品から検出された腸球菌群の型別により，人由来の汚染か動物由来の汚染かをある程度推定出来る.

19. 各種伝染病あるいは食中毒原因菌の主要な媒介者であるネズミの糞便において，腸球

菌群の菌数が大腸菌群のそれより高い傾向にあることは，腸球菌群の食品汚染指標菌としての意義を高めるものであろう．

20. 人及び各種動物の糞便11種中腸球菌群並びに大腸菌群の菌数がともに最高を示したのはイヌであったが，わが国における *Salmonella* の主要な媒介者がイヌであることを考慮すると，大腸菌群とともに腸球菌群を汚染指標菌として検索することは意義が深い．

以上の調査研究成績から腸球菌群の汚染指標菌としての意義は，畜産食品においては大腸菌群と同等に，またある場合にはそれ以上重要であることを認めた．なお上記の諸点から腸球菌群の型別に当っては，Bergey's Manual (1957) の分類型式に *Str. faecium* を付加すべきことを強調したい．

終りに臨み終始御懇篤なる御指導御校閲を賜わった恩師平戸勝七教授及び浜田輔一教授に衷心より感謝するとともに，御協力を戴いた加藤英一助教授，田坂隆美学士並びに教室員各位に謝意を表する．また菌株を御恵与下された北大農学部佐々木酉二教授，同獣医学部清水亀平次助教授，東大農学部尾形学助教授及び農林省家畜衛生試験場波岡茂郎博士の御厚意に深謝する．

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## (X) SUMMARY

Studies on enterococcus group as an indicator of pollution in water, soil, foods and others have been recently reported by many investigators. Though the presence and significance of enterococcus group in various food materials, especially in fish and other marine products had been investigated, there are a few investigations on enterococcus group in milk, meat and their products.

Therefore, this investigation was undertaken to ascertain the significance of enterococcus group as an indicator of pollution in milk, meat and their products. First, the screening media for enterococcus group were examined and the best was determined. Then, using this screening medium, the qualitative and quantitative examinations for enterococcus group in milk, meat and their products and feces of human and various animals were made, on the other hand the counts of coliform bacteria and all living bacteria too were done. Next, the identification, the relationship between the materials inspected and the species of strains detected, the thermoresistance test and the longevity of enterococcus group were studied respectively.

The results obtained are summarized as follows:

1. When the screening medium for enterococcus group is used, the content of sodium azide ($NaN_3$) in the medium must be adjusted from 0.02 % to 0.025% according as the grade of contamination in the inspected materials. But, according as the concentration of $NaN_3$ become thick, it is necessary to notice that growth of enterococcus group is inhibited (Tables 3 and 4).

2. In the distribution of enterococcus group and coliforms in milk, meat and their products, both organisms are detected at nearly 100% and the number of coliform bacteria was predominant in raw materials, but in the heat-treated milk products enterococcus group bacteria was dominant. Enterococcus group are positive in all samples of milk powder, whereas coliforms are negative (Tables 6,7,9～17).

3. Distribution of enterococcus group was 100% in 60 human and 75 animal feces (Tables 20,23 and 24).

4. Of 968 streptococcal strains isolated from milk, meat and their products and feces, 101 were classified as *Str. faecalis*, 100 as *Str. faecalis* var. *liquefaciens*, 3 as *Str. faecalis* var. *zymogenes*, 438 as *Str. faecium*, 93 as *Str. durans*, 138 as unidentified enterococci, 21 as *Str. bovis* and 74 as unidentified streptococci (Table 30).

5. Of 873 strains of enterococcus group, 438 strains were *Str. faecium*; *Str. faecium* occupied the half of strains of enterococcus group.

6. The frequency of detection of *Str. faecalis* and its varieties was greater than the one of *Str. faecium* in raw milk and meat, but in heat-treated foods *Str. faecium* was superior (Table 32).

7. The strains of *Str. faecium* originated from powdered milk were divided into 7 subtypes by their fermentative reaction to carbohydrates. Moreover, it is noted that a definite subtype was detected in the milk powder obtained from a definite dairy plant (Tables 34 and 35).

8. In human and animal feces, the M.P.N. of coliform bacteria was generally greater than the one of enterococcus group bacteria without reference to individual or race or species of human and animals (Tables 20～22). The M.P.N. of enterococcus group bacteria in human feces was far greater than the one of bovine (Tables 23 and 24).

9. In comparison the species of enterococci detected from human feces with the one from animal feces, *Str. faecium* was highly positive in the latter, especially in bovine. In the case of human feces, however, *Str. faecalis* and its varieties as well as *Str. faecium* were isolated to the same grade (Table 33).

10. When the thermoresistance tests of *Str. faecalis*, *Str. faecalis* var. *liquefaciens* and *Str. faecium* were tested under the various conditions, the thermoresistance of *Str. faecium* was the most stable, *Str. faecalis* var. *liquefaciens* was the most labile and *Str. faecalis* was in the midst of these two. From these results, it was experimentally proved that there is a difference of species of enterococci detected between raw and heat-treated foods (Tables 36 and 37).

11. When the longevities of enterococcus group and coliforms were compared with samples of butter, feces and inoculated skim milk being preserved at various temperature, it was found that the longevity of enterococcus group was longer than the one of coliforms. Such result was always obtained without reference to the number of enterococcus group bacteria or coliform bacteria included (Tables 38～40 and 42).

12. Enterococcus group in powdered milk preserved at room temperature survived at least for 15～17 months after manufacture (Table 41).

13. It is the most suitable measure to know how food materials are contaminative that the estimations of number of living bacteria, coliforms and enterococcus group in food materials are performed at the same time.

14. In such milk products as the positive rate of enterococcus group is higher than the one of coliforms, it will be found that the inspection of enterococcus group is better than the one of coliforms for ascertaining the grade of cleanliness of food materials.

15. If the enterococcus group was detected from dairy products such as condensed milk and processed cheese, an insufficient pasteurization or a recontamination after pasteurization are suspected.

16. In preservative foods, for example in certain milk products, it is worthy to survey the presence of enterococcus group as an indicator of pollution, because enterococcus group is usally survival longer than coliforms in foods preserved.

17. In such heat-treated foods as milk powder, it may be possible that the grade of heating or the recontamination after pasteurization is presumed by typing of enterococci detected.

18. As there is a difference of species between the strains derived from human feces and the strains from animal feces, when the typing of enterococci isolated from various foods is made, it may be presumed which the contamination of food originated from human or animal feces.

19. In rats' feces, the M.P.N. of enterococcus group bacteria was generally larger than the M.P.N. of coliform bacteria (Table 20).

20. Both M.P.N. of enterococcus group bacteria and coliform bacteria in dogs' feces were found to be the highest in all 11 kinds of human and animal feces surveyed (Text-fig. 1).

From the above-mentioned results, it was found that the significance of enterococcus group as an indicator of pollution is equal to the one of coliforms, in some instances the significance of enterococcus group is even superior to the coliforms. Also, the author believe that *Str. faecium* is worthy to be shared one species among enterococcus group.

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# 広島大学水畜産学部教官業績目録

(昭和34年1月——昭和35年12月)
(広島大学水畜産学部紀要に掲載されたものは除く)

# List of Papers by the Staff of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, Published during January, 1959 to December, 1960.

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